Life History Strategies of Diadromous and Landlocked Populations of the Spotted Galaxias, *Galaxias truttaceus* Valenciennes in Tasmania

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I hereby declare that this thesis contains no material which has been accepted for the award of any degree or diploma in any university and that, to the best of my knowledge and belief, the thesis contains no copy or paraphrase of material previously published or written by another person, except where due reference is made in the text.

ABSTRACT

A field study was carried out over a period of 20 months to investigate variation in life history traits of the spotted galaxias, *Galaxias truttaceus*. A comparison was made between two landlocked lacustrine populations from the Central Plateau of Tasmania and two riverine diadromous populations from the Tasman Peninsula in south-east Tasmania.

Univariate and multivariate analyses of serial counts and morphometric measurements revealed variation among populations in numbers of fin rays, gill rakers and measurements associated with fins. Differences were more pronounced between fish from different habitat types (creeks versus lakes) than within habitat types.

Gonadal development of both sexes of fish from all localities commenced at the beginning of summer (December). Riverine fish spawned at the end of autumn (May), however, lacustrine fish did not spawn until early spring (September). Temperature is thought to be a major factor influencing the delay in spawning of lacustrine fish. The gonads of lacustrine females continued to grow after the riverine fish had spawned and a comparison of fish prior to spawning revealed a greater investment in reproduction in lacustrine females than riverine females in terms of weight of gonad relative to body weight. From the conversion of proximate constituents to energy values, reproductive investment in terms of the relative energy devoted to the gonad produced a similar result.

Greater somatic fat (and therefore energy) reserves were accumulated by lacustrine fish over summer than riverine fish and greater depletion of these reserves occurred during gonadal maturation of lacustrine fish. At peak levels, larger amounts of fat were incorporated into ovaries of lacustrine females than in those of riverine females. Ovaries generally possessed a larger percentage of fat than testes while testes had a greater percentage of water, protein and ash.

The relatively greater reproductive investment demonstrated by lacustrine fish was not apportioned in the same manner between lacustrine populations. Females from one population produced larger eggs whilst in females from the other population the fecundity increased at a greater rate with body size, in one year of the study. An analysis of life history traits within the galaxiid family affirms the existence of two distinct life history strategies. Diadromous galaxiids spawn before winter, have small eggs and produce large number of eggs, probably related to the overall larger body size of this group. Totally freshwater galaxiids generally spawn after winter, have larger eggs and produce smaller numbers of eggs, which may reflect the overall smaller body size of this group. Although the spawning times of *G. truttaceus* from different habitats were consistent with those found in galaxiid species, egg size, fecundity and body size were not.

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CHAPTER 1 GENERAL INTRODUCTION

It is now well established that life history traits are subject to natural selection. A considerable body of empirical evidence also suggests that for a particular organism a suite of life history traits may act together as a coadapted unit to maximise fitness in different environments (Stearns, 1976, 1983, 1984; Trendall, 1982; Brown, 1983; Hutchings and Morris, 1985). Several studies have attempted to elucidate the nature of trade-offs between such traits as reproductive investment and lifespan (Calow and Woollhead, 1977; Dean, 1981; Tallamy and Denno, 1982), growth and reproduction (Reznick, 1982), present and future reproductive investment (Reznick, 1982) and offspring size and number (Smith and Fretwell, 1974; Wilbur, 1977; Wootton, 1984). Few, however, have succeeded in demonstrating causal relationships between pairs of traits.

The first formal attempt to combine a suite of life history traits into a coadapted unit has been variously attributed to Dobzhansky (1950), Cole (1954) and MacArthur and Wilson (1967). MacArthur and Wilson (1967) and Pianka (1970), postulated two generalisations. They suggested that populations or species experiencing high rates of natural increase (r), in environments where mortality is predominately determined by density-independent factors, will exhibit life history traits such as rapid growth to early maturity, small adult body size, high reproductive investment and short life span. This type of species or population has been termed 'r-selected' (Pianka, 1970) and is used to describe such taxa as mice, rabbits, aphids and many other invertebrate taxa where populations increase rapidly and then crash. These groups of organisms characteristically occupy temporary or unpredictable habitats. The second type of population or species is one which experiences low rates of natural increase and which exists at or near the carrying capacity of its environment (K). This group of organisms often occurs in environments where mortality is mainly determined by density-dependent factors and shows such life history traits as slow growth, maturity at a greater age, a larger adult body size, small reproductive investment and a long life-span. These 'K-selected' organisms are exemplified by many large vertebrate species which maintain populations at approximately equilibrium and often occupy stable or predictable habitats.

The concept of r- and K-selection has lost support recently with criticism being levelled at its deterministic nature and its lack of flexibility (Wilbur, *et al.*, 1974; Tinkle and Hadley, 1975; Stearns, 1977; Hart and Begon, 1982). Although supporters do still emerge (Boyce, 1984), an alternative hypothesis to r- and K-selection proposed by Murphy (1968) and refined by Schaffer (1974) and Stearns (1977, 1980) is 'bet-hedging', based on age-specific mortality. According to this concept, if the survival of juveniles within a population or species is low or unpredictable, then an individual which breeds over a number of seasons (iteroparity), with a small reproductive investment at each breeding, will increase the chance of at least some offspring surviving to maturity. On the other hand if the survival of adults is low or unpredictable, so that chances of breeding again are slight, then an individual which indulges in once only reproduction (semelparity), and invests a large amount of energy at breeding, is at an advantage.

Several studies have set out to determine which of these two concepts, r- and K-selection or bet-hedging, predicts the appropriate set of life history traits in particular environmental situations. Most studies to date have failed to fully support the hypotheses, although a number have shown the inadequacies of r- and K-selection (Stearns, 1980; Dunham, 1982).

The basis of most investigations of life history evolution and variation has been reproductive investment. Reproductive investment refers to the investment (whether it be measured in weight or energetic terms) in reproduction and/or offspring relative to investment in the body of the parent or adult. Studies have examined the absolute amount of energy invested in reproduction, changes in reproductive investment with age of the reproducer (Fisher, 1958; Williams, 1966; Schaffer, 1974; Pianka and Parker, 1975) and the way the investment is packaged, i.e. whether small numbers of large offspring or large numbers of small offspring are produced (Smith and Fretwell, 1974; Wilbur, 1977; Wootton, 1984). Studies concerning the latter attempt to determine whether there is a limited amount of energy available for reproduction so that offspring number can only be increased at the expense of offspring size.

Fundamental to the concept of reproductive investment is that available resources contributing to the total energy of an organism are limiting. This implies a trade-off between energy used for reproductive purposes and energy used for maintenance and growth (Wootton, 1979). Furthermore, if reproductive investment is related positively and non-linearly to body size and age, as occurs in many poikilotherms, current reproductive investment (implying reduced growth) is at the expense of future expected reproductive investment due to a smaller overall size of the reproducer (Reznick, 1982).

Studies of life history evolution and variation essentially work at two levels: inter-specific and intra-specific. Those at the former level often investigate closely related species occupying different environments. Those at the latter level examine divergence of life history traits at the level of the population. Studies at both levels attempt to compare life history traits and attribute differences to varying selective pressures.

The advantages of inter-specific comparisons are that the life histories of species can be generalised if it is determined that inter-population variation is minimal relative to inter-specific variation. Furthermore, species occurring in a wide variety of different environments and being under a wide variety of selective forces can be examined. Disadvantages of this approach may be in defining the ancestral life history strategy from which the life histories of derived species diverged, and that the observed traits are optimal solutions to particular environmental problems and are not constrained by phylogenies (Andrews and Rand, 1974; Vitt and Congdon, 1978; Shine, 1980; Vitt and Lacher, 1981; Stearns, 1983, 1984).

An advantage of the intra-specific approach is that the basic species life history strategy can generally be determined. Differences in life history traits between populations can more easily be attributed to specific environmental factors if populations from several different environments are studied (Cummins, 1986). Although the observed differences may be purely ecophenotypic, initially

environmentally induced variation may provide an advantage to individuals having genes for plasticity in life history traits. For example, if a species colonises a different habitat, and breeding at the normal season is not conducive to the survival of adults, those individuals with genes for plasticity in spawning time may be induced to spawn at a different time purely by environmental factors. Poor survivorship of their inflexible conspecifics may lead to an increase in the frequency of genes for breeding season flexibility.

The aim of the present study is to investigate intra-specific, and to a lesser extent, inter-specific life history variation within the Galaxiidae, a Southern Hemisphere family of salmoniform fishes. There are 36 species currently recognised within the Galaxiidae. Twenty species occur in Australia (18 of which are endemic), 13 in New Zealand (11 endemics), four in South America (3 endemics), and one endemic species in each of South Africa and New Caledonia. Tasmania possesses 15 of the 20 Australian galaxiid species and 10 of these are endemic to the island (McDowall, 1970; McDowall, 1978c; McDowall and Frankenberg, 1981). Ecologically, galaxiid fishes have been separated into two groups based on the presence or absence of a marine juvenile phase. The general life history pattern of the diadromous galaxiid group involves both freshwater and marine stages. The adults mature in freshwater, migrate downstream to the lower reaches of rivers to spawn (in some species into estuaries), the newly-hatched larvae are carried out to sea where they spend about four months, before returning to freshwater as what is commonly termed 'whitebait'. The life history pattern of the totally freshwater group does not involve a marine stage. These species spend their entire lives in freshwater, there appears to be little or no spawning migration, and the whitebait juvenile stage is absent.

Benzie (1968a) and McDowall (1970) noted that diadromous galaxiids tend to produce large numbers of relatively small eggs and spawn in late autumn/early winter. On the other hand, totally freshwater galaxiids tend to have smaller numbers of larger eggs and spawn after winter. Furthermore, diadromous galaxiids tend to be larger than totally freshwater galaxiids. Reproductive investment has only rarely been considered in the analysis of individual galaxiid life histories and data are insufficient for a comparison between diadromous and totally freshwater species.

Galaxiids are thought to have evolved from a proto-osmeroid or salmonoid stock and an ongoing argument involves the probable nature of the life history of the galaxiid ancestor; essentially whether it was spent predominately in the sea or in freshwater. Rosen (1974) and Campos (1984) prefer the latter option, suggesting that the distribution shown by the family today is mainly due to vicariant events, the different ancestral stocks resulting from the Mesozoic break up of the Gondwanaland continent. However, McDowall (1970, 1978c, 1983) noted that the species with wider distributions have a marine phase in their life histories, *e.g. Galaxias maculatus* and *G. brevipinnis* are found in mainland Australia, Tasmania, New Zealand and *G. maculatus* is even found in South America (McDowall,1983). He suggests that the current distribution has been mainly due to marine dispersal and that most of the totally freshwater species of galaxiids have evolved from marine dispersed diadromous ancestors.

Several normally diadromous galaxiid species have become landlocked, preventing a marine juvenile phase. Pollard (1971a, b, 1972a, b, 1973, 1974) laid the ground work for the present study in his investigation of the biology and systematics of the landlocked population of *G. maculatus* in Lake Modewarre in Victoria. He found that landlocked *G. maculatus* had shifted its spawning time from before winter as seen in the diadromous riverine form in New Zealand, to late winter/early spring (Pollard, 1971a). Furthermore the maturation schedule had been shifted forward in time about three months. Otherwise, few differences were found in comparison between this lacustrine form and the diadromous form in New Zealand. A comprehensive study of Australian diadromous populations of *G. maculatus* is yet to be carried out for a comparison with Pollard's findings, although current studies demonstrate that Australian diadromous *G. maculatus* may behave similarly to New Zealand diadromous *G. maculatus* (W. Fulton, pers. comm.).

Several landlocked populations of *G. truttaceus* occur in highland lakes of relatively recent origin (20,000 to 9,000 years B.P.) in the Central Plateau region of Tasmania. In addition, populations of lowland diadromous riverine *G. truttaceus* also occur in Tasmania, thus providing an ideal opportunity for comparisons of life history strategies of geographically close populations. Such comparisons also provide an opportunity to examine if the variation between galaxiid species of different life history strategy groups extrapolates to variation within species.

In the current study, the life histories of two populations of riverine diadromous *G. truttaceus* are compared with two populations of lacustrine totally freshwater *G. truttaceus*. Univariate and multivariate techniques are used to determine the extent of morphological divergence between the populations of *G. truttaceus*. Proximate an alyses of the somatic and gonadal tissues have been performed to determine the mechanism by which the life history traits differ, as well as a means whereby reproductive investment can be expressed in energetic terms. A brief statistical analysis of galaxiid life history traits is performed to determine the validity of Benzie's (1968a) and McDowall's (1970) supposition and the results from this analysis will be compared with those of the intra-specific study.

CHAPTER 2 COLLECTING TECHNIQUES AND STUDY SITE DESCRIPTIONS.

2.1 GENERAL METHODS

2.1.1 Study Species

The species of galaxiid fish upon which this study is based is the spotted galaxias, *Galaxias truttaceus* Valenciennes (Fig. 2.1). The species is distributed throughout Tasmania, south-eastern Australia and south-west Western Australia (Fig. 2.2). It occurs mostly in slow-flowing coastal streams, although there are several isolated lacustrine populations in the western Central Plateau of Tasmania. Its closest affinities are with two landlocked species, *G. auratus* and *G. tanycephalus*, also found in lakes on the Central Plateau. These species are thought to be landlocked derivatives of *G. truttaceus* (Johnson, *et al.* 1983).

2.1.2 Study Localities

Location The two creeks from which *G. truttaceus* was sampled were Fortescue Lagoon Creek (FLC) (43° 8' South, 147° 57' East, map reference Storm Bay EN775227) near Fortescue Bay, and Allens Creek (AC) (43° 5' South, 147° 54' East, map reference Storm Bay EN723306) Tarrana. Both are located on the Tasman Peninsula about 100 km south-east of Hobart (Fig. 2.3). The two lakes from which *G. truttaceus* was sampled were Carters Lake (CL) (42° 40' South, 146° 31' East, map reference Meander DP605652) south of Lake Augusta and Isabella Lagoon (IL) (42° 35' South, 146° 29' East, map refence Mersey GD574634) south-east of Lake Ada. Both lakes are located on the western Central Plateau of Tasmania, about 10 km west of Liawenee (Fig. 2.3).

Dimensions of Water Bodies Both creeks are characteristic of streams on the Tasman Peninsula, being narrow and relatively short in length. FLC extends only *c.* 2.5 km from its headwaters to its mouth at Fortescue Bay, whilst AC is *c.* 7 km long. At their widest points the creeks may reach 5 m across, however, for the most part they are only 2 or 3 m in width. The depths of the creeks vary depending upon the amount of flow, although neither has a depth greater than 1.5 m and generally are less than 1 m.

CL and IL are both relatively shallow, with gently curving shorelines. CL has an area of *c.* 30 ha and therefore is considerably larger than IL, which has an area of *c.* 3 ha. The depth of CL varies from less than 1 m around much of the shore, and where all sampling was carried out, to more than 3 m along the western side of the lake. IL is no more than 1.5 m in depth and for the most part is less than 1 m.

Vegetation Terrestrial vegetation of the Tasman Peninsula is dominated by wet sclerophyll communities, with some areas of temperate rainforest (Davies,1965). The riparian vegetation is predominately *Eucalyptus* and lesser trees such as *Acacia, Banksia, Exocarpus, Busaria* and

Fig. 2.1 Photograph of a typical IL *G. truttaceus* (above) and a typical FLC *G. truttaceus* (below) showing differences in the size and number of spots.
Bar equals 10 mm.

Fig. 2.2 Map showing the distribution of *G. truttaceus* within Tasmania and on the mainland of Australia.

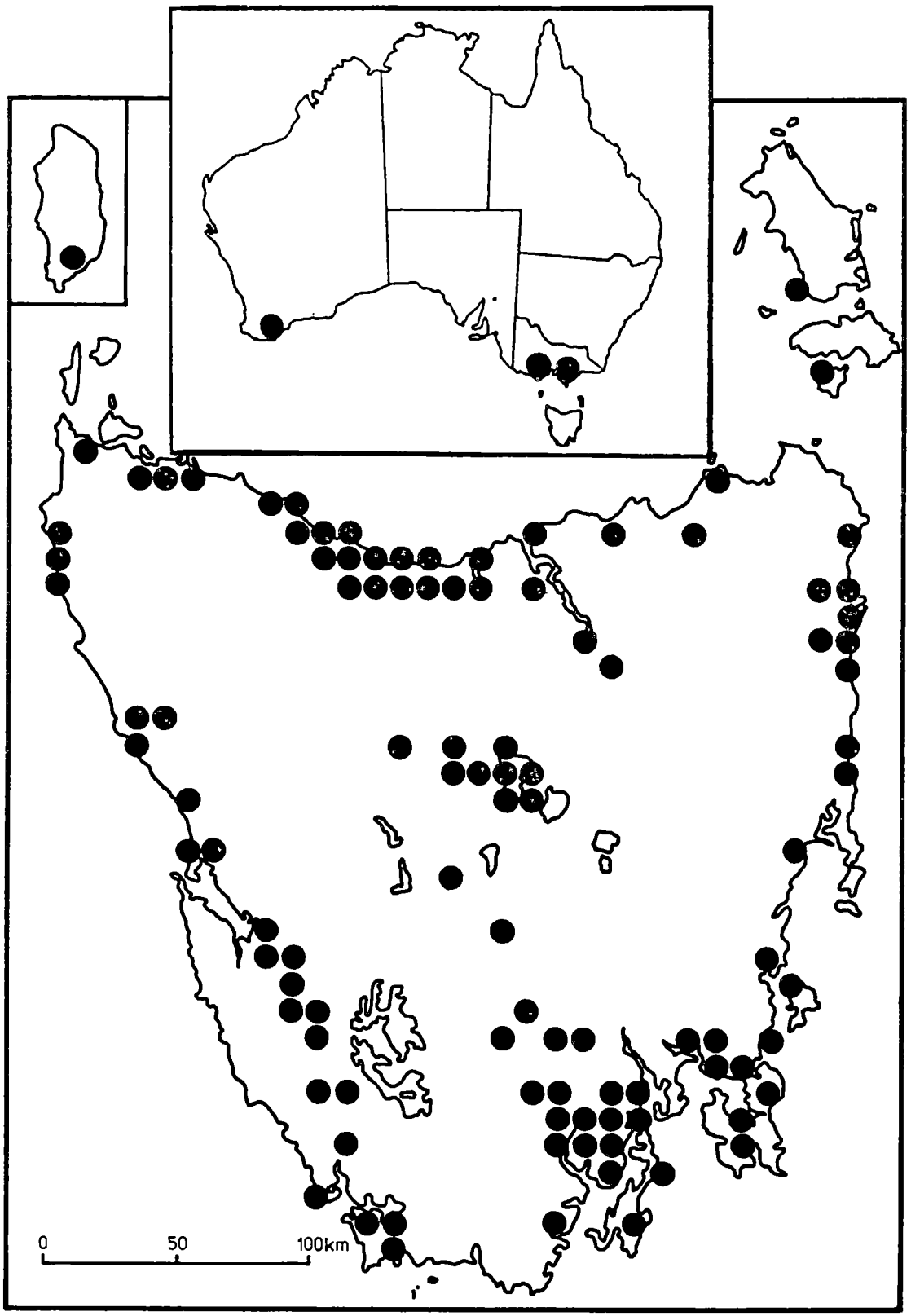
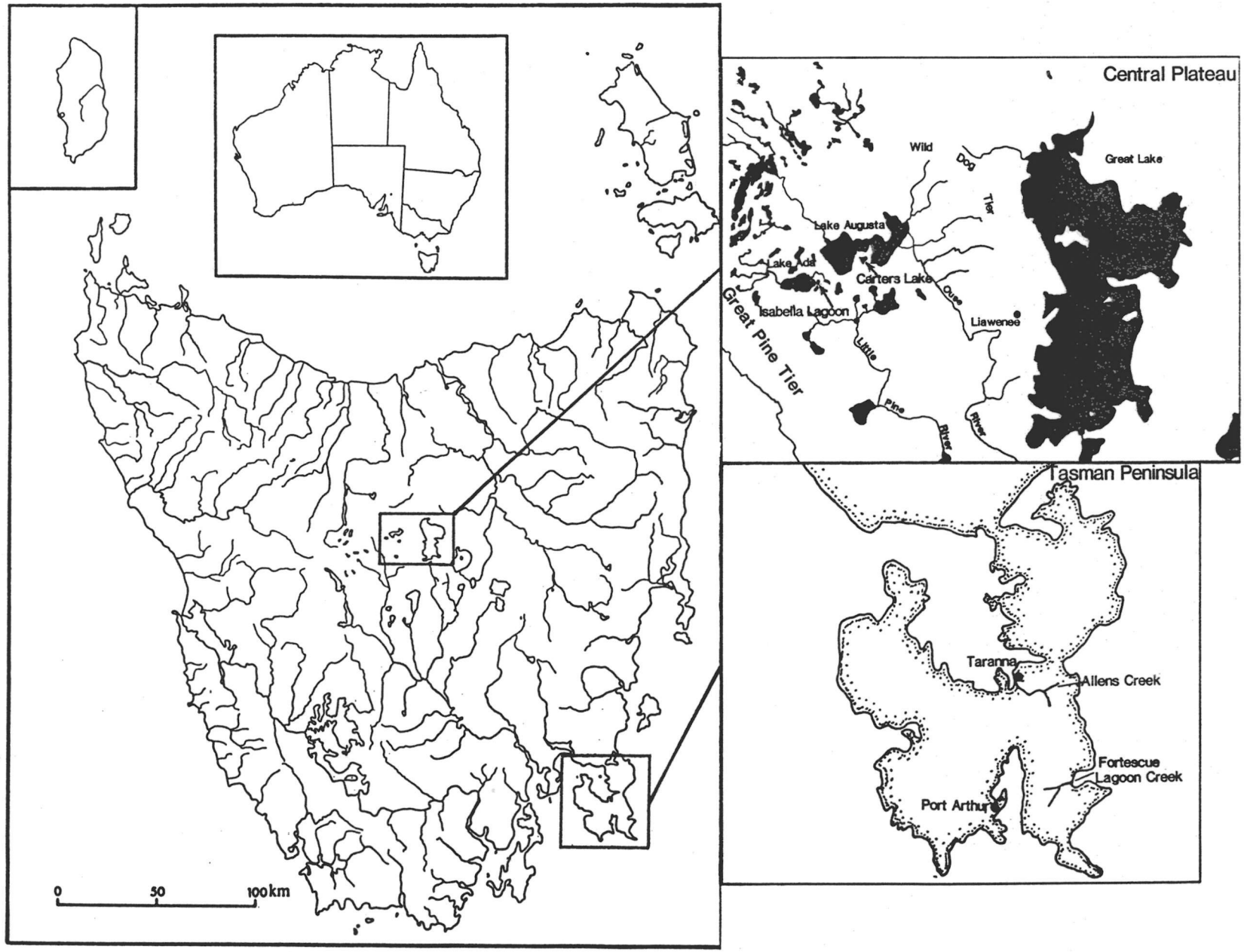

0
50
100km

Fig. 2.3 Maps showing the location of the four study sites, Fortescue Lagoon Creek, Allens Creek, Carters Lake and Isabella Lagoon.


Central Plateau
Wild
Dog
Tier
Great Lake
Lake Augusta
Lake Ada
Carters Lake
Ouse
Isabella Lagoon
Liawenee
Great Pine Tier
Little
Pine
River
River
Tasman Peninsula
Taranna
Allens Creek
Fortescue
Lagoon Creek
Port Arthur
0
50
100km

Casuarina. In rainforest gullies manfern, *Dicksonia antarctica* and myrtle, *Nothofagus cunninghamii,* can be found (Cameron,1981). Aquatic vegetation within the streams is limited to a few species only, such as the water ribbon, *Triglochin procera.*

Terrestrial vegetation of the Western Central Plateau is mostly moorland and austral-montane, consisting mainly of Epacridaceous - proteaceous shrubbery (Davies, 1965). The montane moorland vegetation consists predominately of species of *Orites, Richea, Poa, Helichrysum, Brachycome* and *Olearia,* and cushion plants occur where snow lies for any length of time (Cameron, 1981; Pemberton, 1986).

The aquatic vegetation of the lakes is varied. *Carex* sp. is found along the margins of IL and CL and on an island in the middle of the former and is the most abundant species around the lakes. Submerged macrophytes, such as *Villarsia* sp., *Myriophyllum* sp. and *Callitriche* sp. predominate within the lake itself.

Rainfall, Temperature and Elevation The long-term mean annual rainfall of the Tasman Peninsula is 780 mm and that of the Central Plateau in the Lake Augusta region 1375 mm (Davies, 1965; Pemberton, 1986).

In summer the mean air temperature on the Tasman Peninsula is 14° C, while in summer on the Central Plateau the mean air temperature is 11° C. In winter the mean air temperature is 8° C on the Tasman Peninsula and 4° C on the Central Plateau (Davies, 1965; Pemberton, 1986). FLC is situated approximately 10 m a.s.l. and AC is at approximately 80 m a.s.l., however, the Central Plateau lakes are at both at approximately 1100 m a.s.l.

Geomorphology and the Origins of Central Plateau Lakes Both the Tasman Peninsula and the Central Plateau are essentially composed of Jurassic Dolerite, although there are some regions of Tertiary Basalt, sandstone, mudstone and shales of Permian and Triassic age on the Central Plateau (Jennings and Ahmad, 1957). There are approximately 4000 lakes of various sizes in the north-west Central Plateau (Davies, 1965). Most are of glacial origin, formed when the Pleistocoene ice-cap receded between approximately 20,000 and 9,000 years B.P. The following account is taken largely from Jennings and Ahmad (1957).

The origins of the lakes fall into two broad categories: some were formed by glacial erosion and others by glacial deposition. CL and IL are both of the latter type. There remains some doubt as to the extent of glacial influence, however, it is certain that it extended west of Great Lake over much of the western Central Plateau. The depositional belt reached from Wild Dog Tier south-west past Lakes Augusta and Ada, to Great Pine Tier (Fig. 2.3)

Moraines were formed by moving ice, and by studying their shape and allignment, the direction of movement of ice can be determined. Many lakes, such as Clarence Lagoon, towards the southern edge of the Central Plateau, were simply formed by water courses being dammed by moraine barrages. Other lakes were formed when ice blocks melted in small moraine hummocks. The

formation of the second group was by glacial deposition, the best examples of which lie on the till plain south-east of Lakes Augusta and Ada. This region exhibits a gentle 'sag-and-swell' topography; the swells rising no more than 3-10 m. In several such 'sags' are shallow lakes which have simple rounded shorelines, such as Rocky Lagoon, Lake Paget and CL. Dolerite boulders line the shores of these lakes. The exact nature of the formation of IL is uncertain.

It is likely that both CL and IL were once part of larger forms of Lake Augusta and Lake Ada respectively, when water levels were higher. From aerial photographs it appears that the shorelines of these two lakes may have once extended beyond their present boundaries (Dr M. Banks, pers. comm.). Both the lakes have outflow in the form of the Ouse River for Lake Augusta, and Little Pine River for Lake Ada; both of these rivers are tributaries of the River Derwent. Upstream migrating fish may have colonised the larger ancestral lakes and become trapped in what are now CL and IL when the water table sank. There is evidence that a dry period occurred after the relatively wet Pleistocoene (Keast, 1959). Therefore, it is probable that the populations of *G. truttaceus* became landlocked during a post-glacial dry period and have remained that way since.

Other Fish Species Listed below are the species of fish other than *G. truttaceus*, which have been recorded from each of the four localities. Data are derived from collections made by the Inland Fisheries Commission of Tasmania, from the present study and from Humphries and White (in press).

Family	Species	Common Name
Fortescue Lagoon Creek		
Geotriidae	*Geotria australis*	pouched lamprey
Anguillidae	*Anguilla australis*	short-finned eel
Galaxiidae	*Galaxias brevipinnis*	climbing galaxias
	G. cleaveri	Tasmanian mudfish
	G. maculatus	common jollytail
Allens Creek:		
Geotriidae	*G. australis*	pouched lamprey
Anguillidae	*A. australis*	short-finned eel
Galaxiidae	*G. brevipinnis*	climbing galaxias
	G. maculatus	common jollytail
Bovichthyidae	*Pseudaphritis urvillii*	sandy
Carters Lake:		
Salmonidae	*Salmo trutta*	brown trout
	S. gairdneri	rainbow trout
Galaxiidae	*G. brevipinnis*	climbing galaxias
	Paragalaxias julianus	julians paragalaxias
Isabella Lagoon:		
	none	

2.1.3 Collection of Fish

Two types of electrofishing equipment were used to collect fish. A generator-powered, bank-mounted electrofisher was normally used, however, a 'Smith-Root Model 11-A' back-pack, battery powered electrofishing unit was employed when large distances or rough terrain had to be covered. The generator-powered electrofisher was powered by a Honda E8000 generator producing an alternating current which was rectified to either 50 or 100 pps direct current. Depending on the conductivity of the water, a current between 0.2 and 1.0 amp was produced. The back-pack electrofisher was usually operated with a direct current of between 50 and 60 pps and between 300 and 600 V, producing a current of 0.2 amp.

A 100 m section of stream was electrofished with the operators working upstream to prevent disturbance of substrate hampering observations of fish. In the lakes, operators worked their way back and forth along the edge of the lake in a zig-zag manner until a sufficient sample of fish was collected.

2.1.4 Measurement and Preservation

Sampling commenced in March 1985 at FLC and CL. Isabella Lagoon was not sampled until May 1985 and AC was sampled from September 1985. Fortescue Lagoon Creek and AC were sampled until June 1986, at which time two breeding seasons of the former hed been covered, and CL and IL were last sampled in October 1986, a month after the second breeding season.

The standard length (SL), the length from the tip of the snout to the end of the caudal peduncle, of all fish collected was measured to the nearest 1 mm using a measuring board. A sample of between 15 and 30 fish, encompassing the range of sizes (see Table 3.2 for sample sizes for each collection), was killed by extended immersion in 2% ethyl p-aminobenzoate ('benzocaine') and fixed and preserved in 10% neutral buffered formalin (Hale, 1965).

2.1.5 Physico-chemical Measurements

At each sampling visit the dissolved oxygen concentration of the water was measured with a 'YSI' model 57 portable oxygen meter and pH was measured colorimetrically using a 'Lovibond' field pH kit. A maximum/minimum thermometer was placed at each study site and read at each visit. Flow records for AC were obtained from the Rivers and Water Supply Commission of Tasmania. Given the similar and adjacent catchments and similar stream morphology of FLC and AC, these records should be applicable to both streams.

2.1.6 Morphometrics and Meristics

The degree of morphological divergence of the two stream and the two lake populations of *G. truttaceus* was investigated by the analysis of morphometric and meristic characters. The scheme of McDowall and Frankenberg (1981) was used to record measurements and counts. All measurements were made with vernier calipers, measuring to the nearest 0.1 mm. Some of the measurements and all

of the meristic counts were made with the aid of a low-power dissecting microscope.

The measurements and meristic counts made are listed in Table 2.1. Sample sizes for morphometric analyses were 67, 30, 43 and 38 for FLC, AC, CL and IL respectively. Meristic counts were checked for homogeneity of variances and analysed by analysis of variance and by the multiple comparisons Tukey test for *a posteriori* comparisons (Zar, 1974), after verifying the lack of correlation of counts with body size. Sample sizes for analysis of meristic counts are given on the appropriate figures.

For the analysis of morphometric measurements, data from each locality was initially treated independently. All variables were checked for homogeneity of variances and logarithmically transformed if necessary; each variable was then separately regressed against SL. Regressions of individual variables against SL were compared between localities using analysis of covariance and checked for homogeneity of slopes and elevations. The analysis of covariance enabled a comparison of fish of different sizes. Those variables for which the slopes of regressions among localities differed significantly were rejected. Variables with homogenous slopes among localities were used in subsequent analyses and are shown in Table 2.1 in bold.

Canonical variate analysis (CVA) was performed on a PrimeB computer using the statistical package 'Genstat'. The residuals from regressions of the 11 morphometric variables (referred to above) and SL were used in the analysis (see Atchley, *et al.*, 1976 for a similar approach), categorising the fish based on locality. The mathematical procedures involved in CVA are described by Blackith and Reyment (1971). The multivariate canonical variate technique allows for a comparison of two or more groups using several variables simultaneously. The advantage of multivariate techniques are that the distinction between groups may often be based on a composite effect of variables rather than on the effects of variables individually. CVA transforms measured variables into a set of 'canonical variables' which are linear combinations of the original variables. The first canonical variable is constructed so that it gives the maximum separation of groups, the second canonical variable provides the next greatest separation of these groups and so on. If the first two canonical variables explain a large percentage of the variation then it is possible to satisfactorally represent the relative positions of the groups in two dimensions based on the first two canonical variables. Calculation of Mahalanobis distances, or the distance between groups, provides information on the likelihood of misclassification of an individual into another group.

2.1.7 General Statistical Analyses

Th Students' t-test was used in the comparison of means, after testing for homogeneity of variances. One way analysis of variance was used to compare more than two means and the multiple comparisons Tukey test employed as an *a posteriori* test for differences between pairs of means.

Least squares regression was used to describe bivariate relationships and regression lines were compared by analysis of covariance (Zar, 1974). Slopes of regression lines were initially tested for differences between groups and if this proved significant the analysis was not taken further. If the

Table 2.1 Morphometric measurements and meristic counts made on fish from FLC, AC, CL and IL. (Morphometric variables in bold are used in analyses)

Morphometric measurements	
LCF-Length to Caudal Fork	HL- Head Length
SL-Standard Length	HW- Head Width
BDV-Body Depth at Vent	HD- Head Depth
DCP-Depth of Caudal Peduncle	**SNL- Snout Length**
LCP-Length of Caudal Peduncle	POHL- Post-orbital Head Length
PreD-Pre-dorsal Length	**IOW- Inter-orbital Width**
PreA-Pre-anal Length	LUJ- Length of Upper Jaw
LDB-Length of Dorsal Base	LLJ- Length of Lower Jaw
LAB-Length of A nal Base	WG- Width of Gape
MLD,MLA-Greatest Length of	
Dorsal and Anal Fins	Meristic Counts
PEC-Pectoral Fin Length	
PEL-Pelvic Fin Length	Dorsal Fin Rays
PrePEL-Pre-pelvic Length	Anal Fin Rays
PECPEL-Pectoral to Pelvic Length	Gill Rakers
PELAN-Pelvic to Anal Length	

slopes of regressions were not significantly different elevations were compared. Data were logarithmically transformed, or if percentages, arcsin transformed, where appropriate to ensure homogeneity of variances. Where fluctuations in particular variables have been followed throughout the year and significant relationships existed between body size and these variables, a value for a standard fish of 105 mm in length or 20 g in weight has been substituted into the equations. All other values (where no relationships exist between particular variables and body size) are means of the total data sets and are compared using Student's t-test or analysis of variance. Although estimated values from regression equations are presented in figures, comparisons of regression lines have been by analysis of covariance (ANCOVA). Estimated values from regression equations and means are never compared statistically. Standard errors of estimated values were calculated from:

$$SE = \sqrt{s^2x.y\,[1/n + (\bar{X}-X)^2 / \Sigma x^2]}$$ (Zar, 1974).

2.2 RESULTS AND DISCUSSION

2.2.1 Physico-chemical Measurements

The pH and dissolved oxygen concentration of the lakes fluctuated more than the streams (Fig. 2.4). Creek water was generally saturated with oxygen, never falling below 10 mg/l, while lake water fell to *c.* 7 mg/l over spring and summer. Oxygen concentrations were dependent on water temperatures (Fig. 2.5) and the amount of flow in the streams. Temperatures in streams were more stable than in lakes due to the forested catchments of the former probably acting as insulators. Temperatures in the lakes fell to 0° C over winter and the water was frozen for some weeks of both years of study, while stream temperatures never fell below 4° C. On average, lakes had higher maximum temperatures over summer and lower minimum temperatures over winter. The large differences between maximum and minimum temperatures in the lakes are indicative of large within month variations and probably result largely from the shallowness of these habitats.

2.2.2 Morphometrics and Meristics

From the analysis of the meristic characters it can be seen that lake fish had smaller numbers of dorsal (Fig. 2.6b) and anal fin rays (Fig. 2.6c) than creek fish, but more gill rakers (Fig. 2.6a, Table 2.2). The maximum number of dorsal rays was 11 for creek fish, however, the maximum for lake fish was only 10. Similarly, the maximum number of anal rays for creek fish exceeded that of lake fish. Although the mean number of gill rakers for IL fish was not very much greater than for FLC and AC fish, CL fish showed a large difference. Differences in the number of dorsal fin rays and gill rakers between fish from CL and IL also indicates a greater variation between lakes than between creeks.

In his analysis of morphological variation in diadromous and landlocked populations of *G. maculatus*, McDowall (1972) found a similar situation to that demonstrated here for *G. truttaceus*.

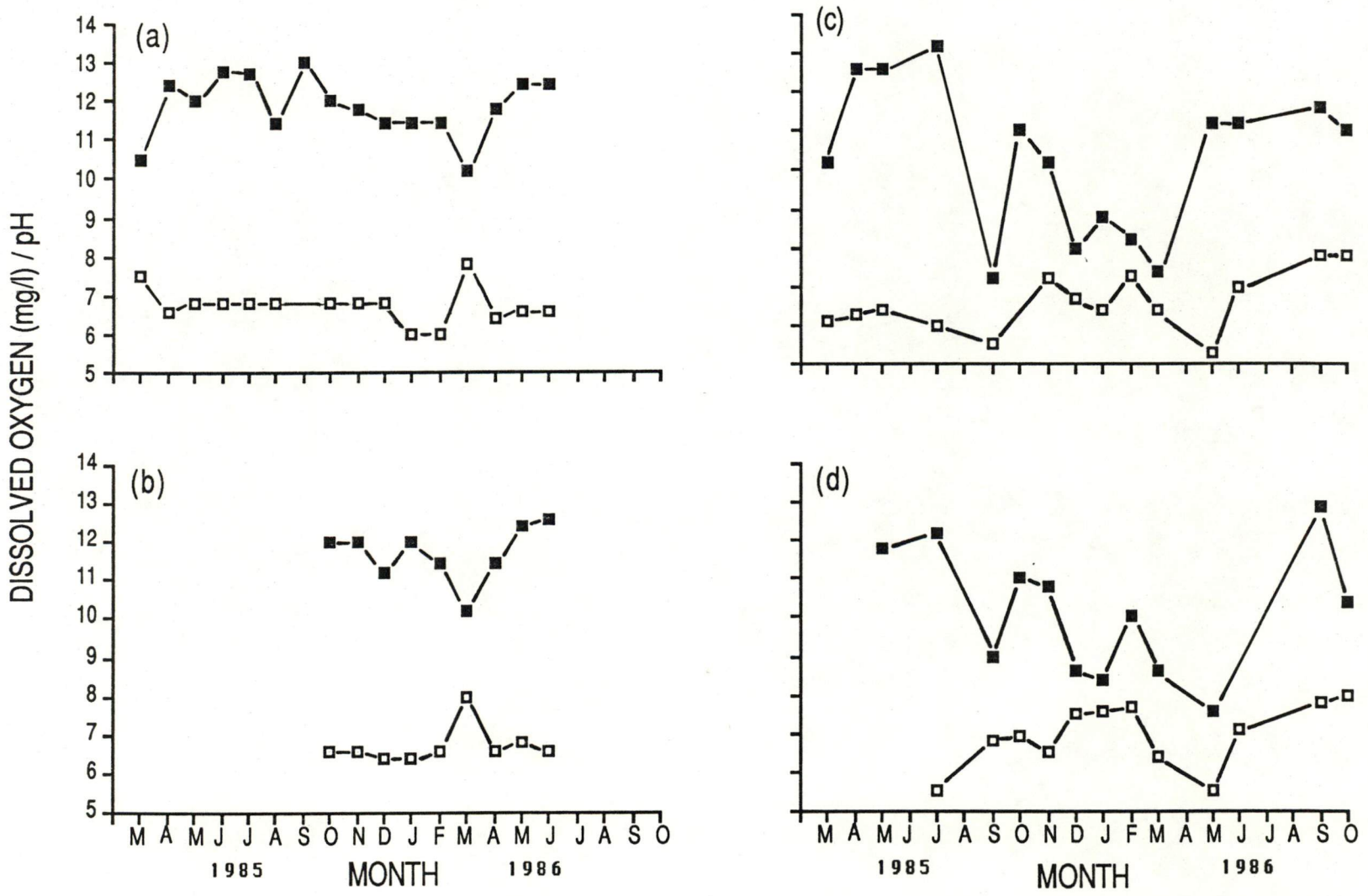


Fig. 2.4 Dissolved oxygen and pH values for water from (a) FLC, (b) AC, (c) CL and (d) IL during the period of March 1985 to October 1986.

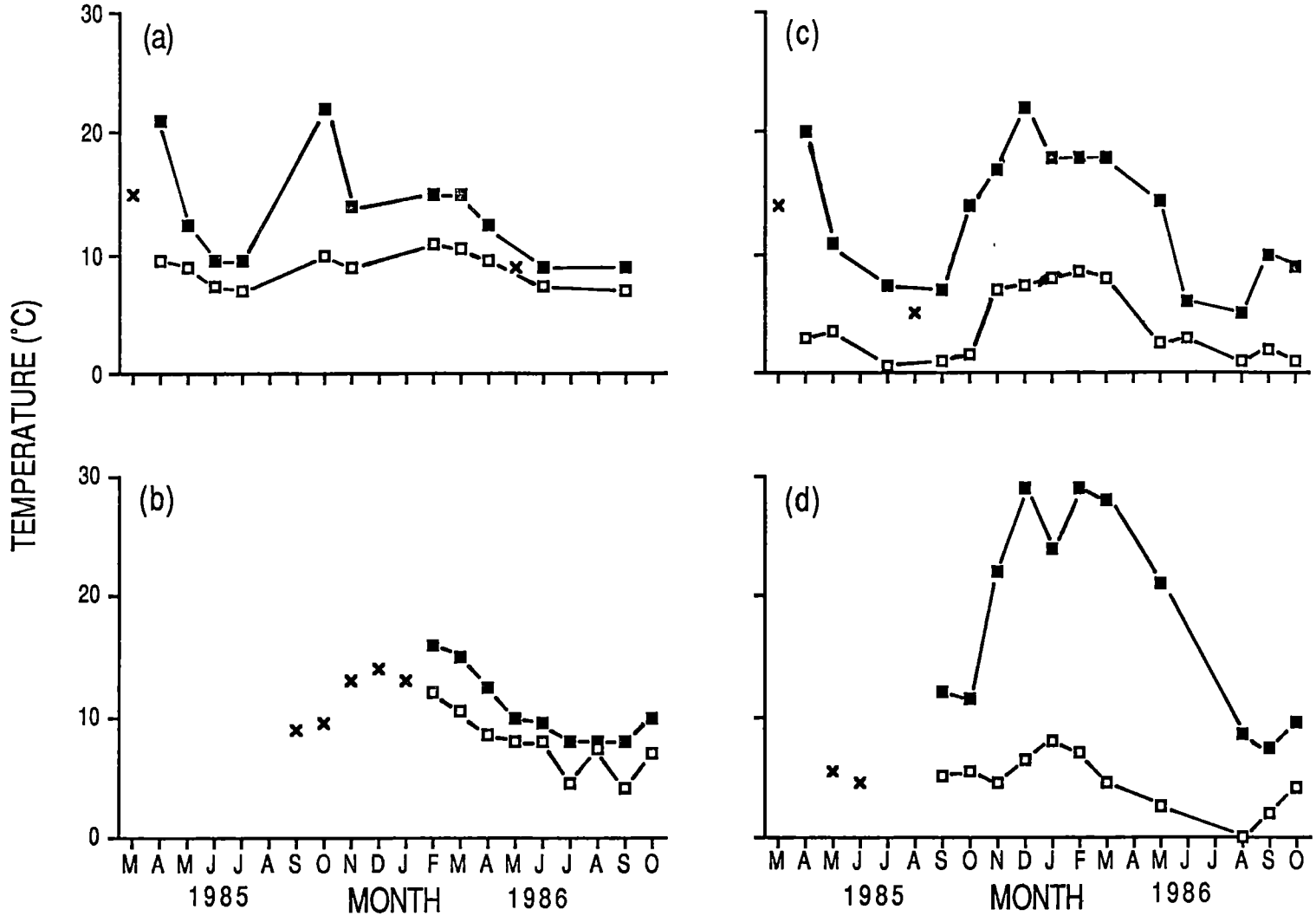


Fig. 2.5 Maximum (closed squares) and minimum (open squares) water temperatures at (a) FLC, (b) AC, (c) CL and (d) IL from March 1985 to October 1986. Crosses idicate readings taken on day of sampling trips.

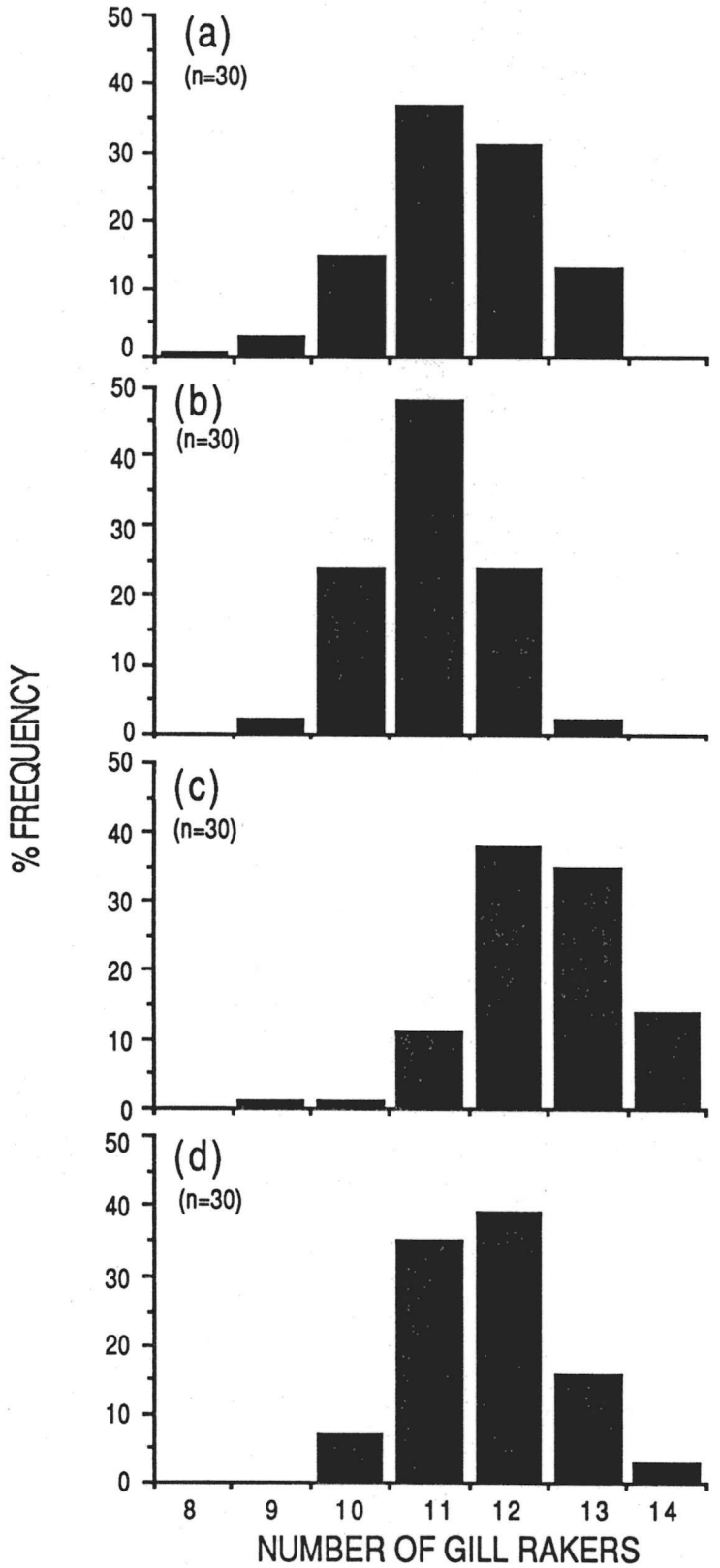


Fig. 2.6.a Percentage frequency of numbers of gill rakers for *G. truttaceus* collected from (a) FLC, (b) AC, (c) CL and (d) IL.

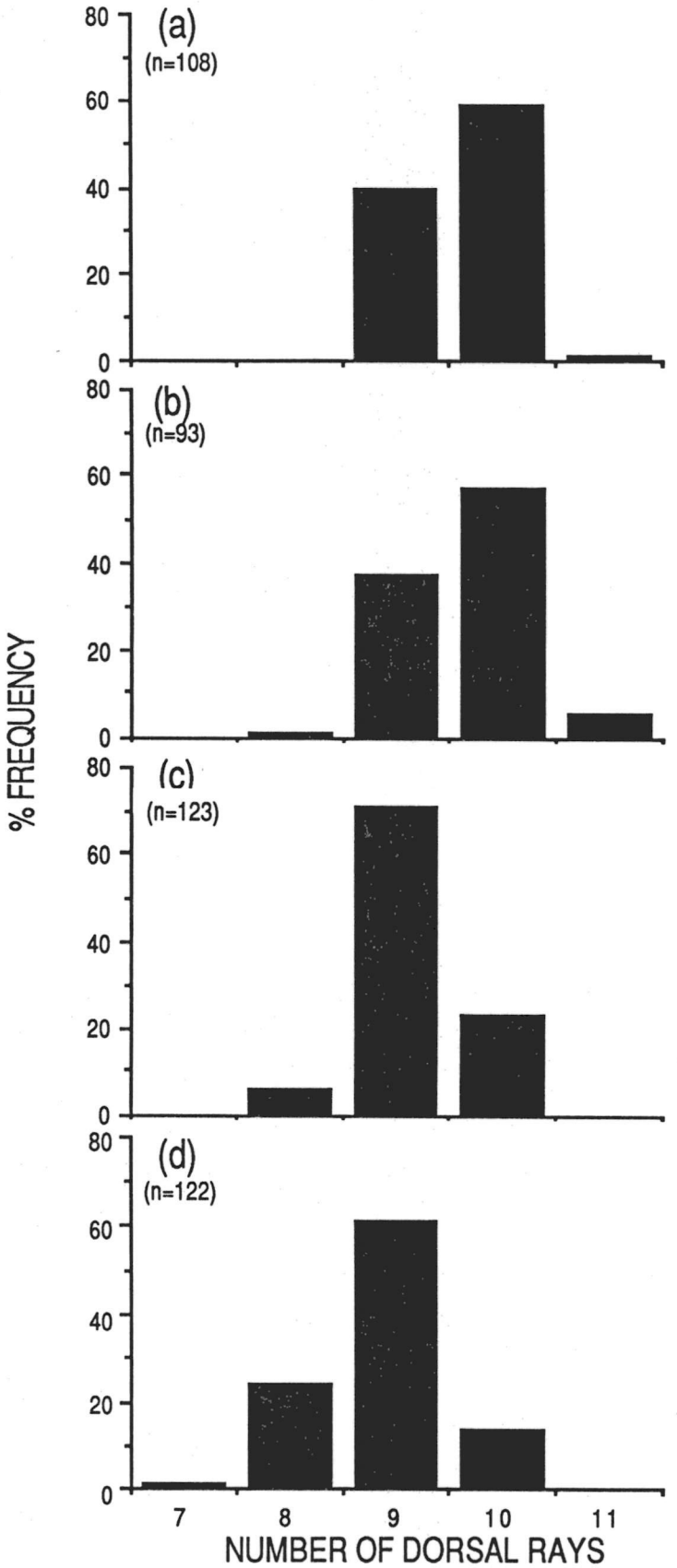


Fig. 2.6.b Percentage frequency of numbers of dorsal rays for *G. truttaceus* from (a) FLC, (b) AC, (c) CL and (d) IL.

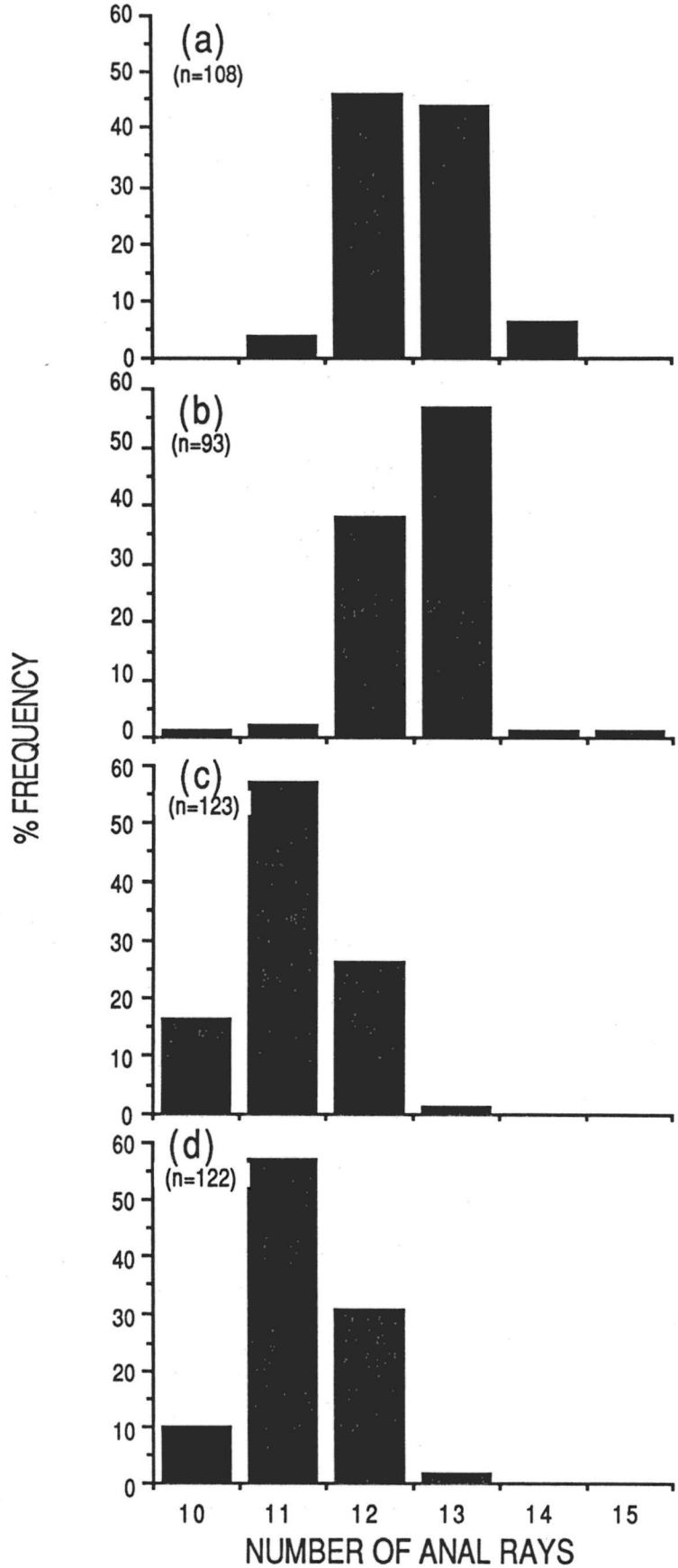


Fig. 2.6.c Percentage frequency of numbers of anal rays for *G. truttaceus* from (a) FLC, (b) AC, (c) CL and (d) IL.

Table 2.2 Mean and standard errors for numbers of dorsal rays, anal rays and gill rakers for fish from FLC, AC, CL and IL. Localities connected by lines are not significantly different from each other.

	Locality				
	FLC	AC	CL	IL	ANOVA
Dorsal Rays					
$\bar{x}$	9.61	9.67	9.16	8.89	df=3,445
SE	0.05	0.06	0.05	0.06	F=48.04
n	108	93	123	122	p<0.0001
Anal Rays					
$\bar{x}$	12.52	12.58	11.11	11.26	df=3,445
SE	0.06	0.07	0.06	0.06	F=154.9
n	108	93	123	122	p<0.0001
Gill Rakers					
$\bar{x}$	11.33	11.00	12.46	11.75	df=3,29
SE	0.10	0.08	0.09	0.08	F=49.9
n	30	30	30	30	p<0.01

Table 2.3 Percentage variation and cumulative variation explained by the first three canonical variables and the loadings (latent vectors) for the standardised morphometric measurements.

	Canonical Variate		
	1	2	3
Percentage of variation explained	73.4	19.8	6.7
Cumulative percentage variation	73.4	93.2	99.9
Variables		Loadings	
LCF	-1.7340	-16.1576	-5.0370
DCP	2.2449	0.6736	-1.8249
LCP	-0.7089	-2.9840	-7.4244
PRD	-6.8762	23.7461	34.8256
PRA	-26.5760	-22.4653	-11.2640
LAB	8.0777	-3.9044	0.6018
MLD	5.3993	-3.8069	4.4360
PEL	0.6023	-9.6070	5.9949
PrePEL	-1.5386	-0.6006	0.2559
PECPEL	0.2683	-0.6115	0.1009
SNL	3.1981	2.7922	-5.1206
IOW	6.0070	11.9186	-4.8399

The mean number of gill rakers in Australian lacustrine populations of *G. maculatus* was greater than the diadromous mean and there was also a tendency for anal and dorsal rays to be reduced in number in the former. The Australian lakes from which McDowall (1972) collected fish are all lowland (114 to 137 m a.s.l.), vary in salinity (1.87 to 7.67 ppt), shallow and considered to be warm. This suggests that similar morphological changes may occur under different environmental conditions, but that the lacustrine habitat may influence characters similarly. It is important to note, however, that both lacustrine and diadromous populations of *G. maculatus* from South America showed little variation in meristic counts (McDowall, 1972). McDowall (1972) has suggested that the greater numbers and increased length of gill rakers of lacustrine *G. maculatus* are adaptive and reflect a transfer from a predominately benthic diet to a planktonic one.

The variation explained by the first canonical variate in the analysis of morphometric variables was 73.4% and the first three variates explained 99.9% of the variance (Table 2.3). The centroids and individual points for the first two canonical variates are plotted in Figure 2.7 and determination of Mahalanobis' distances between centroids revealed that all pair-wise comparisons were significantly different (Table 2.4). The first canonical variate separated the lake populations from the stream populations and the second variate showed some within habitat (*i.e.* within lake and within stream) separation occurring. Morphologies of fish were separated on the first canonical variate by contrasting the pre-anal fin length (**PreA**) and secondarily the length of the anal fin base (**LAB**), with the pre-dorsal fin length (**PreD**), the inter-orbital width (**IOW**) and the maximum length of the dorsal fin (**MLD**) (Table 2.3). The second canonical variate separated fish largely by contrasting pre-anal fin length, length to caudal fork (**LCF**) and pelvic fin length (**PEL**) with pre-dorsal fin length and inter-orbital width (Table 2.3). It is evident that the separation of groups is based predominately on fin characters and associated measurements; an occurrence also shown for the analysis of fin ray counts. An overall value of 84% was obtained for the classification of fish into correct groups (Table 2.5). It can be seen that the percentage of incorrect classifications into groups of different habitat types (stream or lake) was much lower than incorrect classifications into groups of the same habitat.

From the above analysis it is evident that there is variation both between and within habitat types. Variation in fin morphology and vertebrae number appears to be widespread in the Galaxiidae and has been the major source of contention in the systematics of this group (McDowall, 1972). The relative positions of the dorsal and anal fins provide a basis for the separation of the genera *Galaxias*, *Galaxiella* and *Paragalxias* (McDowall and Frankenberg, 1981; Johnson, *et al.*, 1983). Western Australian and Great Lake populations of *G. truttaceus* show morphometric and meristic differences from diadromous populations (McDowall and Frankenberg, 1981). McDowall and Frankenberg (1981) suggest that these differences are related to the absence of a marine juvenile phase in the lacustrine forms resulting in a change in the environment in which larval development takes place. McDowall (1970) attributes some character differences in *G. brevipinnis* in New Zealand to temperature, but also states that those populations of *G. brevipinnis* which are truly landlocked are prevented from interbreeding with diadromous conspecifics.

Table 2.4 Mahalanobis' distances (D-values) for the pairwise comparisons of localities (FLC, AC, CL and IL). * = significant at $p<0.01$

	Values of D			
FLC	0.00			
AC	2.37*	0.00		
CL	3.72*	3.92*	0.00	
IL	4.30*	3.81*	2.30*	0.00
	FLC	AC	CL	IL

Table 2.5 Percentage of individuals from FLC, AC, CL and IL classified into categories based on locality.

		Classification locality			
		FLC	AC	CL	IL
Actual locality	FLC	86.6	7.5	5.9	0
	AC	10	86.7	3.3	0
	CL	2.3	2.3	79.1	16.3
	IL	0	2.6	13.2	84.2
	Mean percentage of correct classifications.		84.2		

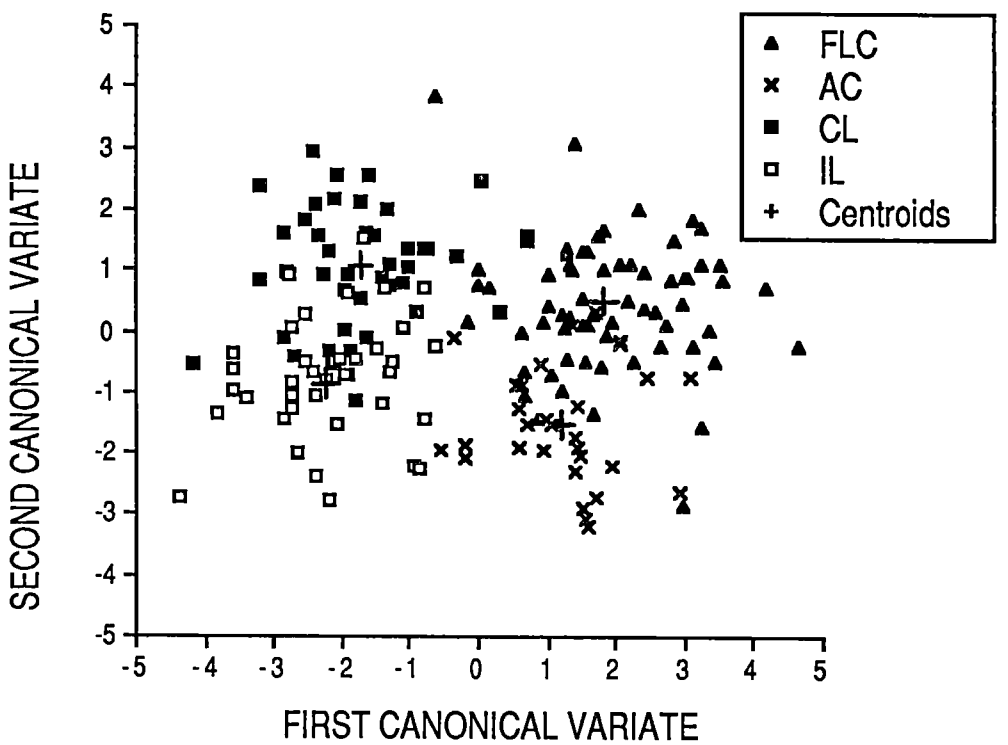


Fig. 2.7 Plot of values for the first and second canonical variates from the canonical variate analysis of size corrected morphometric variables for fish from FLC, AC, CL and IL.

The clear grouping of stream *G. truttaceus* and lacustrine *G. truttaceus* along the first canonical variate either suggests that a factor peculiar to the lentic environment is exerting an influence or that founder effects have played a part in shaping the morphologies of landlocked populations. McDowall (1972) suggests that in a comparison of diadromous and landlocked populations of fish, if the variation between the two groups is no greater than between two diadromous populations, then this can probably be interpreted as simply *'natural variation within the species'*. As indicated by the first canonical variate, there was greater morphological variation between the diadromous and lacustrine populations than between the two diadromous populations or between the two lacustrine populations in the present study.

Further comparisons of galaxiid geographical isolates is needed to assess the nature of the variation found in the present study and ideally, biochemical, ecological as well as further morphological analyses should be performed. Landlocked populations of *G. truttaceus* in Tasmania may provide an ideal opportunity for such analyses.

CHAPTER 3 LIFE HISTORY OF *GALAXIAS TRUTTACEUS*

3.1 INTRODUCTION

Galaxias truttaceus is one of the larger galaxiid species, with fish over 130 mm not uncommon. Although the life history of this species is largely unknown, some aspects of its biology have been investigated.

There are several races of *G. truttaceus* which have been given species or subspecies status in the past, such as *G. scopus* (Stokell) from Clarke Island in Bass Strait and *G. truttaceus hesperius* (Whitley, 1944) from Western Australia. Whitely (1944) suggested that differences in fin ray counts and fin morphology of the Western Australian form necessitated the establishment of a subspecies. However, Andrews (1976) and McDowall and Frankenberg (1981) indicated that considerable variation in the species precluded any formal taxonomic status and suggested that isolated populations were merely ecological races.

Scott (1941) gave a detailed account of the species' distribution, salinity tolerance and migrations as whitebait, and suggested that normally stream-dwelling adults might move out *'to sea on a falling tide'*. He also hypothesised that *G. truttaceus* might spawn in brackish water, or even in the sea, and specimens collected suggested an autumn breeding. He reported that a female of 113 mm standard length possessed 5643 eggs which were between 1.0 and 1.3 mm in diameter, however, his attempts to hatch out artificially fertilised eggs failed. Blackburn (1950) recorded *G. truttaceus* as a major component of the annual whitebait run in Tasmania, while Andrews (1976) noted the occurrence of a troglodytic form of *G. truttaceus* which differed only in colouration from stream-dwelling forms.

The life histories of some other galaxiids are known in greater detail, most of the studies having been performed on New Zealand species. Burnet (1965), Benzie (1968b) and McDowall (1968) have described the life history of stream-dwelling *G. maculatus*, while Pollard (1971a, b,1972a, b, 1973, 1974) compared the biology of this form to that of a landlocked population in Lake Modewarre, Victoria. The life history of another diadromous galaxiid, *G. fasciatus*, was studied by Hopkins (1979b) and spawning was observed by Mitchell and Penlington (1982). The biology of some totally freshwater species such as *Galaxias vulgaris* (Benzie, 1968a; Cadwallader, 1976), *Neochanna apoda* (Eldon, 1978), *Neochanna burrowsius* (Eldon, 1979a,b), *Galaxias olidus (Fletcher 1979)*, *Paragalaxias dissimilis* and *P. eleotroides* (Fulton, 1982) and *Galaxiella pusilla* (Backhouse and Vanner, 1978; Humphries, 1986) have been investigated to varying degrees.

Several galaxiid species, including *G. truttaceus*, *G. brevipinnis*, *G. fasciatus*, and *G. maculatus*, have both stream-dwelling diadromous populations and landlocked totally freshwater populations (McDowall, 1972). In his study of the Lake Modewarre population of *G. maculatus*, Pollard (1971a) reported features of the life history that were different from those of the New Zealand diadromous form. Diadromous *G. maculatus* spawn in autumn amongst flooded vegetation in estuaries and the eggs develop out of water and hatch at the next spring tide. The larvae are then washed out to sea and return some months later as whitebait (McDowall, 1968). Pollard (1971a)

found that landlocked *G. maculatus* spawned in late winter/early spring in freshwater in flooded tributaries of Lake Modewarre, the eggs also experienced a period of extra-aquatic development and they hatched during the next flood, the larvae being carried out into the lake. The spring spawning landlocked *G. maculatus* began to mature later than the autumn spawning diadromous *G. maculatus* but most gonadal development was completed before winter in both forms. Final maturation in landlocked fish is thought to occur at the time of movement into flooded streams.

A number of lacustrine galaxiid species, including *G. gracilis* in New Zealand and *G. auratus*, *G. tanycephalus*, *G. pedderensis* and *G. johnstoni* in Tasmania are thought to be derivatives of ancestral diadromous species (McDowall, 1970; Johnson, *et al.*, 1983; McDowall and Frankenberg, 1981). As stated previously, diadromous galaxiids generally have large numbers of small eggs and breed in autumn, while totally freshwater species generally have smaller numbers of larger eggs and breed in late winter/early spring. Differences between life history strategies in the two groups of galaxiids have been attributed to the occurrence or lack of a juvenile marine phase (Benzie, 1968a; McDowall, 1970), as well as the degree of effort taken in courtship, nest-building, spawning and fertilisation of eggs (Cadwallader, 1976).

This chapter presents results of the study of the life history of two stream-dwelling diadromous and two landlocked totally freshwater populations of *G. truttaceus* in Tasmania. The extent to which life histories of landlocked forms have diverged from presumably ancestral diadromous forms is assessed and the nature of, and variation in, gonadal development, reproductive investment, spawning, early development and growth are discussed.

3.2 MATERIALS AND METHODS

3.2.1 Sex Determination and Sex Ratios

The shape of the genital papilla was recorded for all sampled fish after December 1985. Preserved fish were mostly sexed by macroscopic examination of the gonads but viewing of a wet mount of gonadal tissue under high power (X400) was sometimes necessary to determine the sex of juvenile fish.

The ratio of males to females for all preserved fish was calculated for each month and for various size classes. The Chi-square statistic was used to test whether these proportions differed significantly from 1:1.

3.2.2 Size and Age of Fish

The standard length of all preserved fish, was measured to the nearest 0.1 mm with vernier calipers. This measure was used in all calculations involving fish length.

Each fish was aged by examination of the growth rings within its sacculus otolith (Williams and Bedford, 1974). Otoliths were removed from fish by making a transverse vertical incision in the top of the head, midway between the operculum and the eye. Each otolith was split vertically through its

nucleus with a scalpel blade and the split halves were heated slowly in a candle flame until they turned light brown. The otolith halves were mounted, split side up, in silicon sealant on a microscope slide (Fig. 3.1) and were viewed under a dissecting microscope, using incident light against a black background. The number of light and dark (opaque and hyaline respectively) rings on the otoliths were counted and an estimate of the age made assuming a pair of rings to represent one years growth. Any otoliths which proved difficult or ambiguous to read, were discarded. Because of the uncertainty as to where one ring began and where it finished no attempt was made to measure the distance between successive rings for back-calculation purposes.

Several methods for validating ageing techniques have been suggested by Williams and Bedford (1974) and two of these were used in the present study. Several fish known to be in their first year were maintained in the laboratory under ambient light and temperature conditions and the presence of light and dark rings in their otoliths noted after one year. In the field, the increase in size of a strong 0+ cohort was followed and the nature of the rings on the otoliths of these fish observed throughout the year. The ageing technique, at least for 0+ and 1+ fish, proved accurate and it is assumed that the nature of the otolith growth did not change with age.

3.2.3. Gonadal Development

The maturity stage of each fish was estimated by macroscopic examination of the gonads. Maturity stages were modified after Pollard (1971a), using six stages rather than his seven (Table 3.1) Pollard's Stage II was not used as the distinction between the adjacent stages was unclear. Distinguishing between Stage IV (mature) and Stage V (ripe) of preserved and fresh fish relied on the behaviour of gonadal contents when the gonad was gently squeezed at its most posterior extremity. If milt or eggs were extruded from the cloaca, then the fish was considered ripe. The gonad was removed and weighed to the nearest 0.1 mg.

3.2.4 Reproductive Investment

Several measures of reproductive investment have been used by fish biologists, the most popular being the gonadosomatic ratio or GSR. This index is simply the weight of gonad divided by the weight of the body expressed as a percentage, and suffers from statistical difficulties shown by all ratios constructed to remove the effect of size (Atchley *et al.*, 1976; de Vlaming *et al.*, 1982) and is clearly inadequate should there be a correlation between GSR and body size (Mills, *et al.*, 1983). A more rigorous way of expressing reproductive investment was used in the present study. The relationship of gonad weight and body weight was analysed by least squares regression. Comparisons between regression lines were then made using analysis of covariance to remove the effect of different sized fish. To follow fluctuations in gonad weights throughout the year a value for a standard weight fish was substituted into the regression equation. This estimated weight of gonad was then expressed as a percentage of the standard body weight and an 'adjusted' GSR value obtained. In the present study a standard of 105 mm and 20 g, which represents the mean size of

Fig. 3.1 Photograph showing an example of a split otolith mounted on a glass slide.

reproductive females (stage III through VI) from all four localities, was used as the standard value in all substitution calculations involving regression of a life history parameter with body size. If no significant relationship existed between gonad weight and body weight, the mean value for all fish in the particular group was used in analyses. Comparisons were not made between months where relationships existed between gonad weight and body weight and where mean values were used.

Table 3.1 Identification of Maturity Stages

Stage I -	Gonads threadlike and thin, colourless, sexes only distinguishable by microscopic examination.
Stage II -	Gonads thickening, opaque, sexes distinguishable macroscopically.
Stage III -	Gonads enlarged. Testes opaque and whitish. Ovaries opaque and yellowish.
Stage IV (Mature) -	Gonads fill most or all of body cavity. Testes creamy white. Ovaries, yellow, eggs large.
Stage V (Ripe) -	Gonad fills or distends body cavity. Testes white, smooth. Ovaries yellow, eggs large, translucent. Gonadal products extruded by pressure.
Stage VI (Spent)-	Gonads thin and flaccid, translucent, residual gonadal products sometimes present.

Regressions of gonad weight against body weight for individual maturity stages, for each sex and for each locality were compared by analysis of covariance between the two years of the study, 1985 and 1986, to determine whether any inter-annual differences existed. No differences were found and therefore data for the two years have been pooled in analyses of regressions of gonad weight with body weight for fish at different maturity stages.

3.2.5 Egg Size and Fecundity

The diameters of between 30 and 50 randomly selected developing oocytes from each female *G. truttaceus* were measured using an ocular micrometer. This was not done for fish at maturity stage VI as connective tissue obscured the view of oogonia. The volume of ripe oocytes was calculated by assuming them to be spherical. Oocyte diameters were regressed against body length and, where the line fit was significant, the standard length was substituted into the equation. Where the fit was non-significant, the mean ± standard error of oocyte diameters for all females from that particular group was used in analyses. Regression lines were compared using analysis of covariance

and one way analysis of variance was used to test for the difference between means.

In order to determine fecundities a subsample was taken from the ovaries of stage IV and V females and weighed to 0.1 mg. Oocytes were separated and counted in a plankton sorting tray and the total number of oocytes in the ovary was calculated by proportioning. Fecundities were regressed against body lengths separately for each population, and where no differences existed between the fecundity versus body length regressions among localities, common equations were calculated.

3.2.6 Collection of Eggs and Larvae

The eggs of most galaxiids are adhesive and therefore extensive searches of the creeks were undertaken to locate the deposition sites of eggs. All conceivable sites were investigated, including boulders, gravel and live and dead vegetation. As *G. truttaceus* eggs might have also been swept by strong currents from deposition sites in the creeks, drift nets with a mesh size of 0.5 mm were placed in FLC and AC in each of six weeks subsequent to spawning. Nets were set so that the majority of water flowed through them, and were placed at intervals along the length of the streams in order to determine from which section the eggs originated. In each stream net #1 was placed just below the normal sampling site, net #2 was placed *c.* 50 m above brackish water, net #3 was placed at the junction of freshwater and brackish water and occasionally a fourth net was placed inbetween nets #1 and #2. The nets were left in place for between 3 and 5 h; the contents were sorted fresh in the laboratory within 12 h of sampling.

Eggs in the lakes were collected by hand by removing clumps of bankside vegetation beneath the water surface. Vegetation, with eggs attached, was taken intact back to the laboratory, placed in aquaria and maintained at *c.*12° C.

Newly hatched larvae of stream fish were collected in a similar manner to that used to collect eggs. Larvae of lake fish were collected with a conical plankton net (mouth diameter of 27 cm, mesh size of 250 μm), which was swept just below the water surface.

3.2.7 Embryonic and Larval Development

The descriptions of development of *G. truttaceus* embryos and larvae are based on collections of eggs from IL on 11 September 1985. The eggs and larvae were maintained in the laboratory at *c.* 12° C, which was *c.* 2° C in excess of the temperature of the lake at this time. Further collections were made to determine the rate of development in the field.

3.2.8 Growth

The collection and measurement of fish for analysis of length frequencies is as described in Sections 2.1.3 and 2.1.4. The sexes could only be distinguished by external secondary sexual characters when near maturity (see Section 3.3.1), and since this was not recognised until late in the present study, sexes have been pooled for analysis of length frequency data. Data have also been grouped seasonally to improve sample sizes in order to allow better interpretation of cohorts.

Length-frequency results were analysed, where possible, by the procedure described by MacDonald and Pitcher (1979), using the computer programme "Mix". This analysis is based on a maximum liklihood statistical procedure by minimising the generalised sum of squares (MacDonald and Pitcher, 1979). It necessitates the initial estimation of proportions, means and standard deviations of the age components. The lengths of most age groups were derived from analysis of length-frequency data; the remaining are means of fish aged by examination of otoliths.

Several models are available to describe the growth in length of animals and the three most commonly used are the logistic, Gompertz and von Bertalanffy (Ricker, 1975). The Gompertz and Von Bertalanffy models differ from the logistic model in that they both show a marked decrease in growth rate in later stages, while the logistic model begins slowly and has faster growth in later stages (Ricklefs, 1967). Most fisheries biologists employ the Von Bertalanffy model to describe growth in length and this was attempted in the present study for *G. truttaceus*. Walford plots have been graphed to determine probable theoretical maximum sizes (L_{∞}) of fish for each population.

3.2.9 Field Trips to Other Lakes

Three lakes additional to CL and IL were sampled once each during the present study. Rocky Lagoon (41° 54' South, 146° 29' East, map reference Mersey DP584633) was sampled in March 1985, Little Blue Lagoon (41° 52' South, 146° 28' East, map reference Mersey DP573652) was sampled in January 1986 and Perched Lake (42° 30' South, 145° 45' East, map reference Olga CN920865) was sampled in April 1986. The first two lakes are situated on the western Central Plateau near to CL and IL. Perched Lake is a lowland lake which rests 17 m above the Gordon River in south-west Tasmania.

3.2.10. Maintenance of Fish in Laboratory

To determine whether variations in life history traits of *G. truttaceus* had a plastic or genetic basis, 0+ fish from FLC and IL were maintained in the laboratory under ambient photoperiod and temperature for one year, until the populations from whence they came spawned. They were initially fed to satiation and the amount of food consumed, as a percentage of body weight, was maintained throughout the year. Fish were fed on dried *Tubifex* and *Daphnia*. When the respective wild populations had spawned the laboratory fish were killed and preserved in 10% neutral buffered formalin. Their sex, length, weight, condition, gonad weight and, if they were females, egg size and number were determined.

3.2.11 Parasites

The occurrence and approximate number of external and internal parasites for all *G. truttaceus* collected were noted. Infestations were classified into four categories: (0) where there were no parasites visible; (1) where there were between one and 10 parasites visible; (2) where there were

between 10 and *c.* 50 parasites visible and (3) where there were more than *c.* 50 parasites visible. The type of parasite was also noted.

3.3 RESULTS

3.3.1 Sex Determination and Sex Ratios.

The sex of fish from maturity stage III to spawning, could be distinguished externally on the basis of the shape of the genital papilla immediately anterior to the cloaca. In maturing females the papilla formed into two lobes (Fig. 3.2a), whilst males possessed a single elongate structure (Fig. 3.2b). After spawning, these characters regressed and sexes were again indistinguishable.

On a monthly basis sex ratios of fish sexed by dissection were generally not significantly different from 1:1 (Table 3.2). The exceptions were at FLC in May in both 1985 and 1986 (X^2=7.2 and 6.92 respectively, $p<0.01$), at AC in October 1985 (X^2=4.8, $p<0.05$) and March and May 1986 (X^2=8.53 and 4.57 respectively, $p<0.05$) and at IL in August and October 1986 (X^2=6.13 and 5.56 respectively, $p<0.01$). In each case there were more females than males.

Differences occurred in the proportions of each sex within particular size groups of fish between habitat types (Table 3.3). There were more females than males in the smallest size class (45-65 mm) of creek fish (FLC: X^2=8.73, $p<0.01$; AC: X^2=4.00, $p<0.05$) but no differences existed for lake fish. There were no differences in numbers, within the size range 65-85 mm, of each sex in creeks, whilst lake males were more abundant at this size than lake females (CL: X^2=4.79, $p<0.05$; IL: X^2=9.00, $p<0.01$). Females were again more numerous for the size range 85-105 mm in creeks (FLC: X^2=6.72, $p<0.01$; AC: X^2=20.6, $p<0.001$) and females were more abundant at FLC, CL and IL for the next size class (X^2=13.06, 27.46, 26.00 respectively, $p<0.001$). In the largest size class (125-145) few fish were caught, except at IL, where only one male was recorded from 46 fish (X^2=42.08, $p<0.001$). Overall, females were more numerous than males at three of the four localities (FLC: X^2=57.54; CL: X^2=16.56; IL: X^2=12.85, all $p<0.001$).

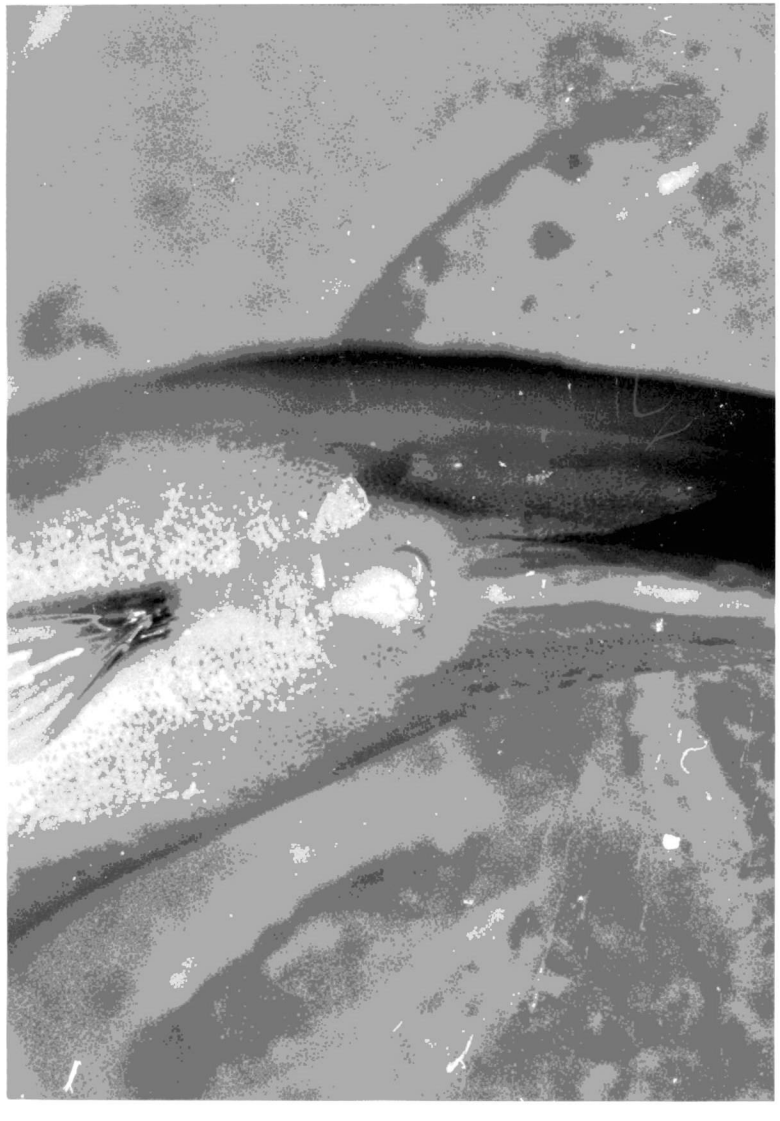
3.3.2 Size and Age of Reproductive Fish

The mean size of stage III to stage VI (reproductive) females was greater than that of stage III to stage VI males at all localities (Fig 3.3; unpaired t-test - FLC: df_{156}, t=5.85, $p<0.001$; AC: df_{37}, t=2.71, $p<0.05$; CL: df_{124}, t=8.39, $p<0.001$; IL: df_{222}, t=10.64, $p<0.01$). The mean size of reproductive females differed among populations (ANOVA - $df_{320,3}$, F=13.36, $p<0.001$), with IL females being larger than AC and FLC females and CL females being larger than FLC females. Furthermore, the mean size of reproductive males among localities was not homogenous (ANOVA - $df_{209,3}$, F=10.73, $p<0.001$). IL males were larger than CL and FLC males. In all cases females were the largest fish collected and at FLC, CL and IL the smallest representatives were males.

The majority of *G. truttaceus* matured at the end of their second year of life. Some males from

Fig. 3.2.a Photograph of the genital papilla of a mature female *G. truttaceus*. Bar represents 1 mm.

Fig. 3.2.b Photograph of the genital papilla of a mature male *G. truttaceus*. Bar represents 1 mm.



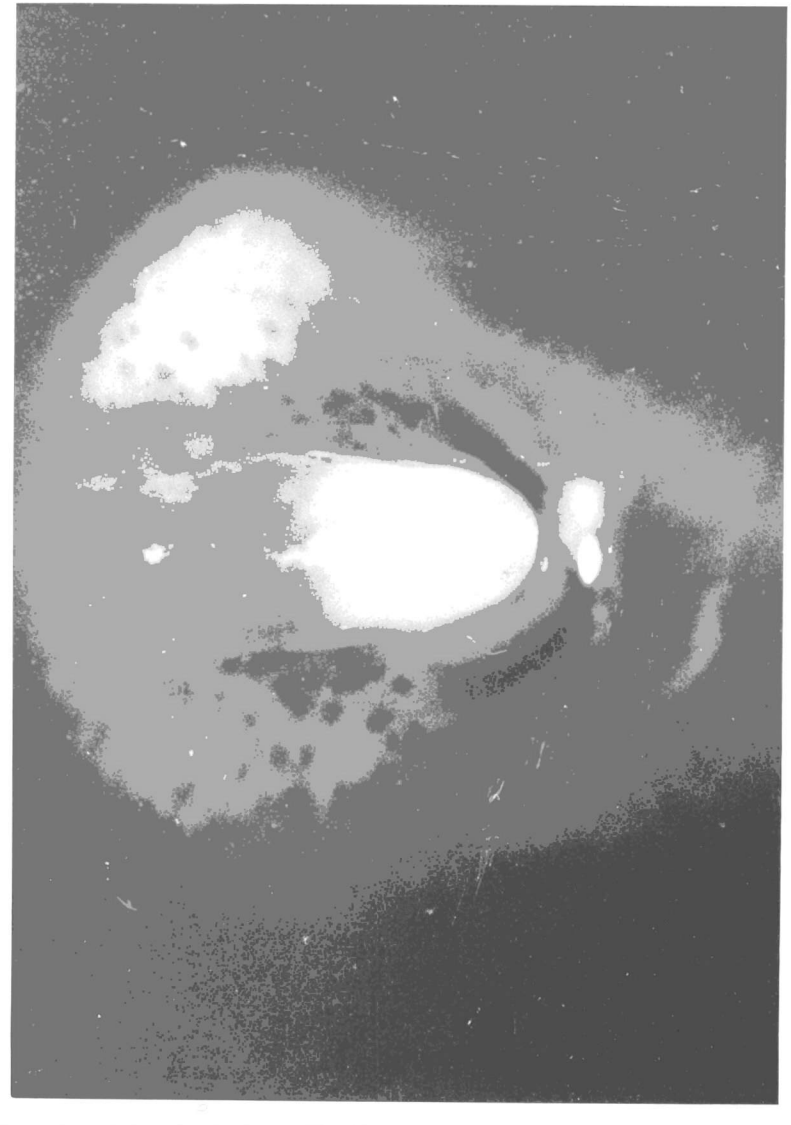


Table 3.2 Monthly sex ratios (female : male) for fish from FLC, AC, CL and IL. (Dashes indicate no sample) * = 0.01< p < 0.05 ; ** = 0.001< p < 0.01

Locality	Month																
	1985									1986							
	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	DEC	FEB	MAR	APRIL	MAY	JUN	AUG	SEP	OCT
FLC	13:11	12:8	16:4**	14:6	11:4	10:5	--	15:9	11:8	8:12	20:10	16:11	18:5**	--	--	--	--
AC	--	--	--	--	--	--	--	21:9*	12:8	13:7	23:7*	16:14	11:3*	--	--	--	--
CL	10:9	7:7	5:5	--	1:1	--	10:4	7:8	12:8	10:9	10:9	--	10:4	7:8	11:7	3:2	4:4
IL	--	--	10:13	--	9:6	--	10:6	7:8	14:6	10:10	8:7	--	8:7	9:6	23:9*	15:11	14:4*

Table 3.3 Sex ratios (female : male) of size groups of fish from FLC, AC, CL and IL. * = $0.01 < p < 0.05$ ** = $0.001 < p < 0.01$; *** = $p < 0.001$

Size range (mm)	Locality			
	FLC	AC	CL	IL
45-65	45:21***	24:12*	23:18	14:16
65-85	44:44	28:26	16:31*	5:20***
85-105	44:22**	33:5***	30:27	40:53
105-125	28:6***	10:05	33:2***	61:16***
125-145	4:0*	2:0	1:0	45:1***
TOTAL	165:53***	97:48**	103:78	165:106**

Table 3.4 Standard lengths of female and male fish at age 2 from FLC, AC, CL and IL. To improve sample sizes values from autumn and winter for creek fish are pooled and values from winter and spring months for lake fish are pooled.

Locality	Female	Male	Significance Between sexes
FLC	78.27±1.18	76.26±1.55	ns
n	21	13	
AC	76.4±3.2	72.3±2.1	ns
n	9	7	
CL	80.6	76.05±1.79	ns
n	1	4	
IL	95.30±1.23	83.66±3.67	$0.001 < p < 0.01$
n	9	12	

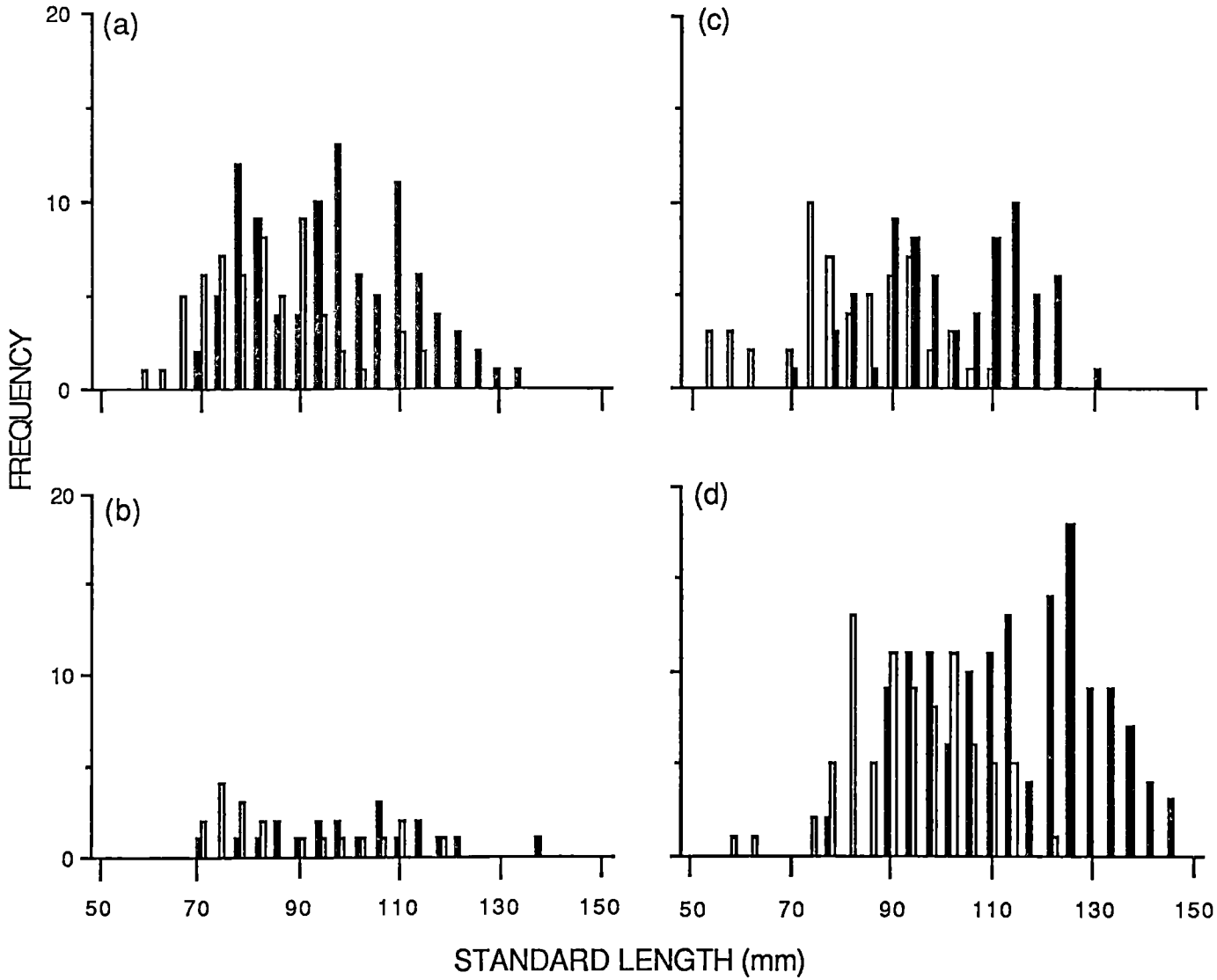


Fig. 3.3 Length-frequency distributions of reproductive (stage III through stage VI) females (closed columns) and males (open columns) from (a) FLC, (b) AC, (c) CL and (d) IL.

CL (n=4) and IL (n=1) matured at 12 mo, however, no females were ever found mature at this age. The mean lengths of fish at age 2 are shown in Table 3.4. The only significant difference in length at age 2 between sexes was at IL, where females were larger than males (unpaired t-test - df_{17}, t=5.88, p<0.01). IL females were of a larger length at age 2 than females from the three other localities, although only one 2 year old CL female was collected at this time (unpaired t-test - FLC/IL: df_{28}, t=8.12, p<0.001; AC/IL: df_{16}, t=11.16, p<0.001) and IL males were larger than FLC and AC males (unpaired t-test - FLC/IL: df_{23}, t=3.99, p<0.001; AC/IL: df_{17}, t=4.55, p<0.001).

3.3.3 Gonadal Development

The gonadal cycles of female and male fish from the four populations are shown in Figs. 3.4.a, d and 3.5.a, d, respectively, in the form of adjusted GSR's plotted against time. The gonads of reproductive fish of both sexes from all localities began developing in early summer (December). The weight of gonads relative to the weight of the body of lake males (Fig. 3.5.d) increased more rapidly than lake females (Fig. 3.4.d) in the initial stages of maturation. By February 1986 CL reproductive males had an adjusted GSR of 12.24 which was significantly greater than that of CL females which had an adjusted GSR of 3.88 (ANCOVA - ns for slopes; for elevations $df_{10,1}$, F=166.63, p<0.001), and IL males had an adjusted GSR of 7.53 which was significantly greater than that of IL females which had an adjusted GSR of 4.47 (ANCOVA - ns for slopes; for elevations $df_{14,1}$, F=4.09, p<0.05). The gonadal cycles of creek fish of both sexes followed a similar pattern (Figs. 3.4.a and 3.5.a).

Creek fish took three months to mature to stage IV (Figs. 3.4.b, c, 3.5.b, c) and they spawned at the end of autumn in May. Some lake fish took only three months to mature (Figs. 3.4.e, f, 3.5.e, f), but all lake fish did not spawn until the beginning of spring (September); nine months after the commencement of maturation. The gonads of lake females continued to increase in weight after May (Fig. 3.4.d). The gonads of males from CL did not increase in relative size after March 1986 and those of IL males, although increasing significantly between March and May (ANCOVA - ns for slopes; for elevations $df_{10,1}$, F=14.36, p<0.01), did not increase after May (Fig. 3.5.d). A month prior to spawning lake females had proportionally larger gonads than FLC females (ANCOVA - FLC/CL: for slopes $df_{16,1}$, F=22.14, p<0.001; FLC/IL: for slopes $df_{26,1}$, F=5.49, p<0.05). Only two ripe females were collected from AC just prior to spawning and so no comparisons are made. No inter-population differences in peak GSR's existed between males from FLC, AC and IL, however, IL males had a significantly larger GSR at their peak than that of CL males (ANCOVA - ns for slopes; for elevations $df_{5,1}$, F=13.55, p<0.05).

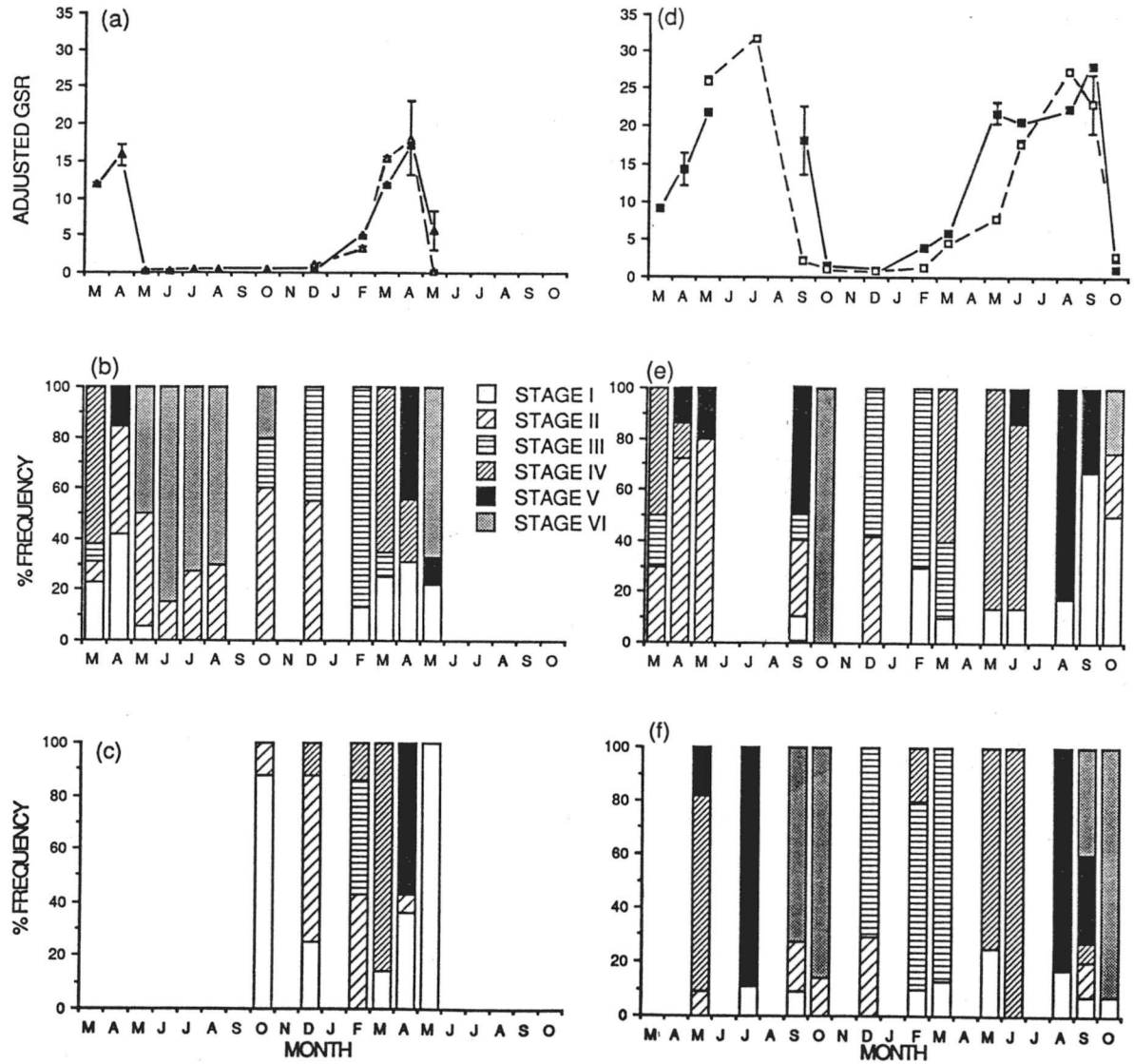


Fig. 3.4 Adjusted gonadosomatic ratios (GSR) ±SE and maturity stages for female *G. truttaceus* from the four localities from March 1985 to October 1986. (a) GSR's for FLC (closed triangles) and AC (open triangles) females; (d) GSR's for CL (open squares) and IL (closed squares) females. Maturity stages for (b) FLC, (c) AC, (e) CL and (f) IL females.

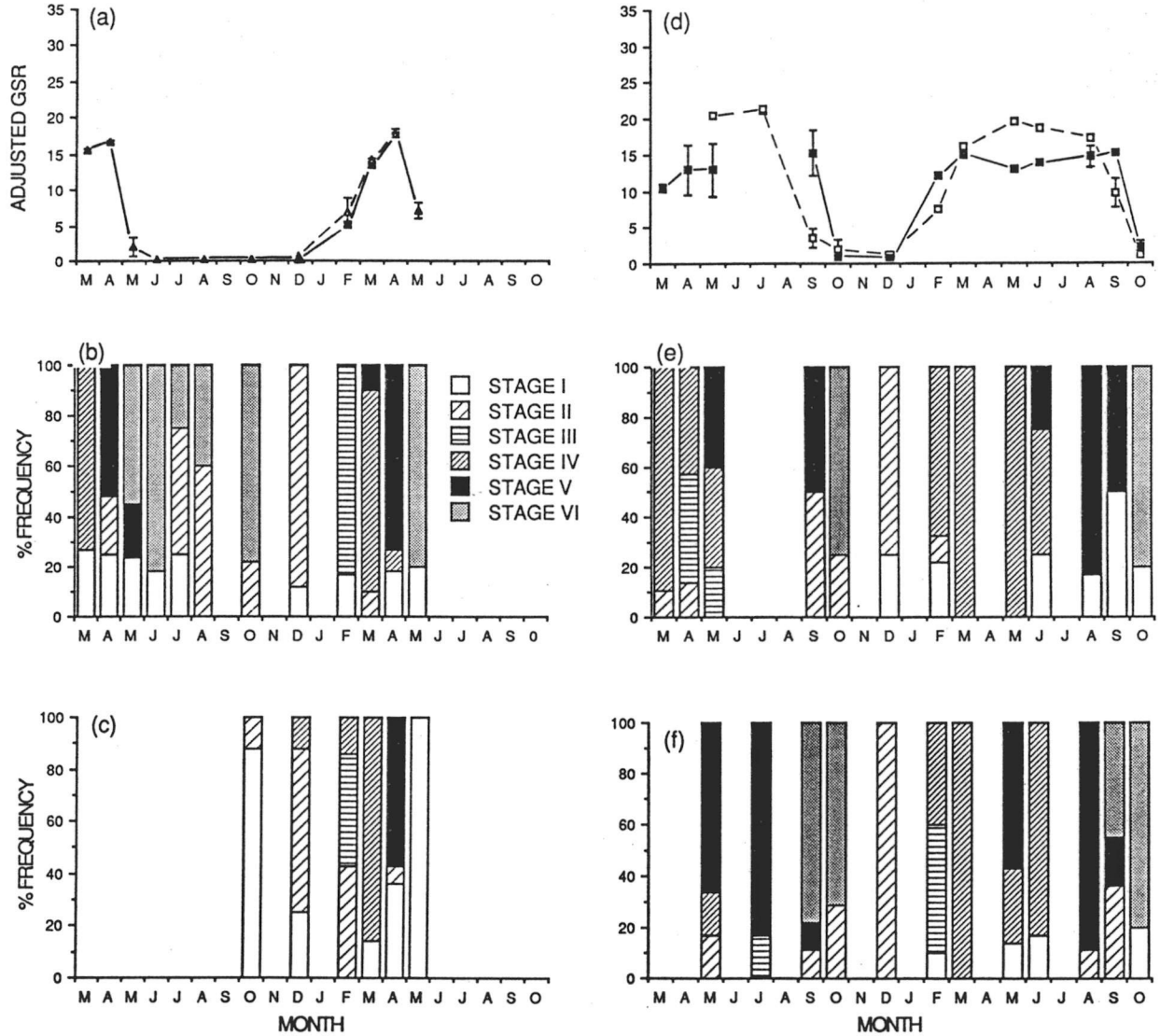


Fig. 3.5 Adjusted gonadosomatic ratios (GSR) ±SE and maturity stages for male *G. truttaceus* from the four localities from March 1985 to October 1986. (a) GSR's for FLC (closed triangles) and AC (open triangles) males; (d) GSR's for CL (open squares) and IL (closed squares) males. Maturity stages for (b) FLC, (c) AC, (e) CL and (f) IL males.

3.3.4 Reproductive Investment

Adjusted GSR's for females at each maturity stage are shown in Table 3.5. There were no differences in adjusted GSR's (as determined from ANCOVA's between gonad weight versus body weight regressions) among localities for females at stages I through III. At stage IV there were differences between lake and creek female adjusted GSR's (ANCOVA - for slopes: $df_{76,1}$, F=31.72, p<0.001) and AC females had a lower adjusted GSR than FLC females (ANCOVA- for slopes $df_{27,1}$, F=24.8, p<0.001). There were between habitat-type (lake versus creek) differences (ANCOVA- for slopes: $df_{71,1}$, F=21.92, p<0.001) but no within habitat-type differences for stage V females. Stage VI females from all localities possessed similar adjusted GSR's.

There were no differences between adjusted GSR's for males from all localities for stage I and stages III through VI (Table 3.6). For adjusted GSR's of males at stage II all pairwise combinations of localities, except AC and CL showed significant differences (ANCOVA- FLC/AC: for slopes $df_{26,1}$, F=15.73, p<0.001; FLC/CL: for slopes $df_{26,1}$, F=4.27, p<0.05; FLC/IL: for slopes $df_{33,1}$, F=39.97, p<0.001; AC/IL: for slopes $df_{25,1}$, F=6.50, p<0.05; CL/IL: for slopes $df_{25,1}$, F=5.20, p<0.05).

Fig. 3.6 shows the gonad weight/body weight regressions for female *G. truttaceus* from the four localities, for the maturity stages at which relationships proved significant. Full regression equations are given in Appendix 1a. At stage IV, CL and IL females had gonad weight/body weight regressions which had significant negative intercepts (CL: t=2.30, p<0.05; IL: t=2.23, p<0.05), whilst the intercepts for FLC and AC females were not significantly different from zero. At stage V the intercepts for gonad weight versus body weight regressions for CL and IL females remained significantly negative (CL: t=3.45, p<0.01; IL: t=2.17, p<0.05) and the intercept for FLC females was again not significantly different from zero. These equations reflect that, as body size increased, GSR's in stage V IL and CL females increased, although this increase was more pronounced for CL females from age 2 to age 3 than for IL females (Table 3.7). Although the intercept for FLC females at stage V was not significant, there was a marginal decrease in GSR with increasing body size. Because body size, expressed as length, was highly correlated with age in *G. truttaceus* (see Section 3.3.9), older CL females invested proportionally more in reproduction than younger females. Females of different ages from FLC and IL invested similar weights of gonad in reproduction. The change in adjusted GSR with age was slight for FLC females (-13%) and IL females (+11%), however, it increased by almost 100% for CL females, from age 2 to age 4 (Table 3.7).

The regressions of gonad weight against body weight for male *G. truttaceus* passed through the origin and therefore male adjusted GSR's changed only slightly with increasing size and age (Fig. 3.7; Appendix 1b). The regressions for FLC and CL males at stage V had marginally negative intercepts, whilst the intercepts at this stage for AC and IL males were marginally positive.

Table 3.5 Adjusted gonadosomatic ratios (GSR) calculated from the substitution of a standard fish of body weight 20 g into regressions of body weight against gonad weight for maturity stages I through VI for females from FLC, AC, CL and IL.(* = mean values due to insignificant regressions)

Stage	Locality			
	FLC	AC	CL	IL
I	0.90±0.052	0.22±0.002	0.32±0.013	0.42±0.130*
II	0.31±0.004	0.35±0.004	0.49±0.012	0.36±0.007
III	3.49±0.181	1.95±0.601*	2.58±0.200	3.48±0.340
IV	13.56±0.115	9.41±0.190	15.11±0.949	21.09±0.480
V	18.23±0.236	20.60±1.440*	24.17±0.237	27.21±0.330
VI	0.43±0.007	-	0.86±0.020	5.30±0.016

Table 3.6 Adjusted gonadosomatic ratios (GSR) ± SE calculated from the substitution of a standard fish of body weight 20 g into regressions of body weight against gonad weight for maturity stages I through VI for males from FLC, AC, CL and IL.(* = mean values due to insignificant regressions)

Stage	Locality			
	FLC	AC	CL	IL
I	0.08±0.004	0.08±0.002	0.34±0.22*	0.29±0.03*
II	0.17±0.002	0.29±0.004	0.28±0.009	0.44±0.005
III	5.65±0.118	5.16±3.03*	4.16±1.27*	5.16±1.20*
IV	13.47±0.116	13.96±1.672*	13.52±0.103	16.23±0.201
V	17.58±0.109	17.94±0.253	15.97±0.211	18.29±0.135
VI	1.10±0.410*	-	1.45±0.57*	6.19±0.004

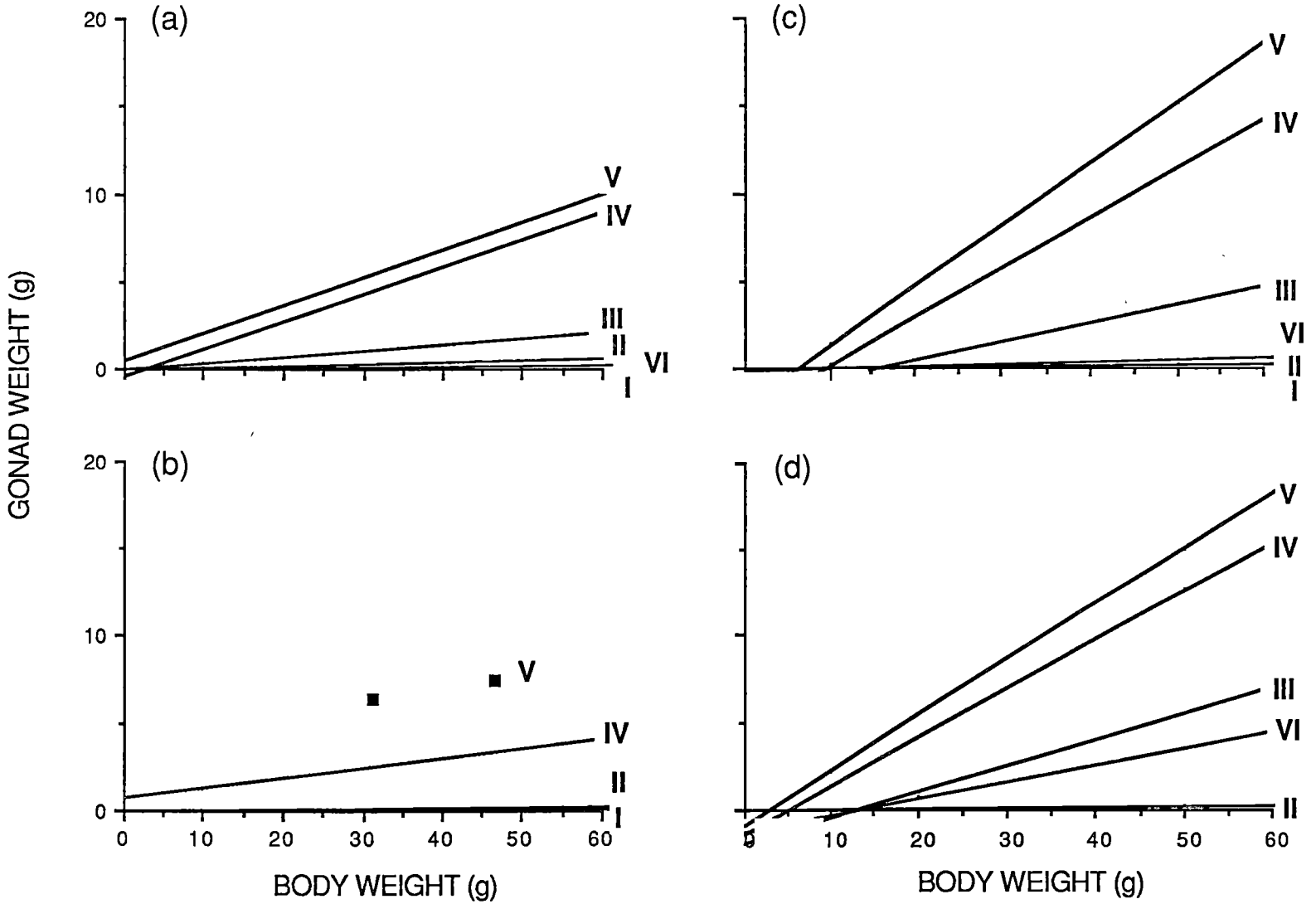


Fig. 3.6 Gonad weight versus body weight regression lines for maturity stages for female*G. truttaceus* from (a) FLC, (b) AC, (c) CL and (d) IL.

Table 3.7 Lengths, weights and gonadosomatic ratios (GSR) for age 2, 3 and 4 females from FLC, CL and IL. GSR values are from substitutions of the body weight of a standard fish of 20 g into regressions of gonad weight against body weight.

	Age (years)		
	2	3	4
FLC			
Length (mm)	80.99	100.30	102.00
Weight (g)	8.71	17.24	18.19
GSR	21.20	18.59	18.45
CL			
Length (mm)	80.56	95.25	100.97
Weight (g)	8.56	14.62	17.61
GSR	10.30	20.34	22.70
IL			
Length (mm)	92.57	99.97	107.59
Weight (g)	13.34	17.06	21.57
GSR	24.89	26.41	27.55

Table 3.9 Diameters of recently fertilised eggs collected from AC on May 20 1986 and from IL on September 3 1986.

Locality	Egg diameter (mm)
AC	1.7, 1.6, 1.7, 1.6, 1.5, 1.6, 1.6
	$\bar{x}\pm SE = 1.61\pm0.07$
IL	1.8, 1.9, 1.9, 1.9, 1.9, 1.9, 1.9, 1.9, 2.0, 1.9, 1.9
	$\bar{x}\pm SE = 1.90\pm0.04$

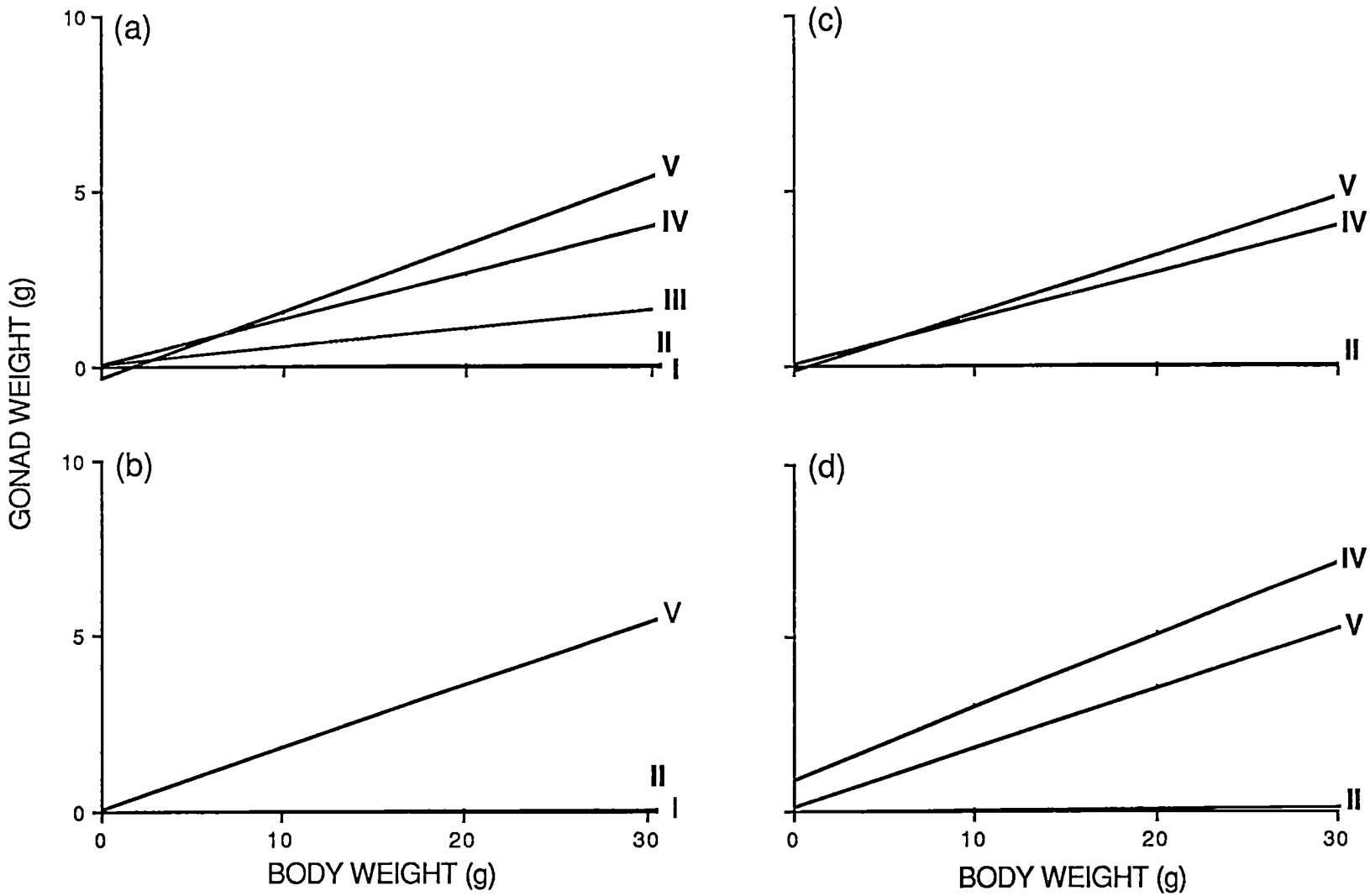


Fig. 3.7 Gonad weight versus body weight regression lines for maturity stages for male*G. truttaceus* from (a) FLC, (b) AC, (c) CL and (d) IL.

3.3.5 Egg Size and Fecundity

Egg diameter began increasing between October and December from its basal size of *c.* 0.15 mm at all localities (Fig. 3.8). FLC eggs reached their maximum ($\bar{x}$±SE) size of 1.27±0.03 mm when fish spawned in 1985 and 1.23±0.02 mm in 1986. Egg diameters of AC fish were at a similar size to FLC eggs one month earlier in 1986. Uncertainty of the time of spawning of fish at AC prevented further comparisons from being made. The size of eggs of CL females did not increase significantly from May to spawning in September, at which time eggs were 1.21±0.02 mm in diameter. Egg sizes of IL fish increased after May 1986, and by September were 1.34±0.01 mm in diameter. Mean egg diameters of IL fish were significantly larger at the time of spawning than those for FLC and CL fish (unpaired t-test - IL/FLC: df_{18}, t=3.36, p<0.01; IL/CL: df_{24}, t=5.89, p<0.001), whilst there were no differences in mean egg diameters between FLC and CL. No variation in mean egg diameter within localities between years was apparent.

Body volume can be a limiting factor in reproductive investment, so egg volume may be a more meaningful description of egg size (Kaplan and Salthe, 1979). The differences between egg sizes of IL females and FLC and CL females are more pronounced if egg volume is used as a measure of egg size (Table 3.8). In 1986 the mean egg diameter of IL females was *c.* 9% larger than the mean egg diameter of FLC females and *c.* 11% larger than for CL females. However, in volumetric terms, the eggs of IL females were *c.* 30% larger than those of FLC females and *c.* 35% larger than the eggs of CL females.

Table 3.8 Egg volumes derived from egg diameters of spawning fish.

Locality	Year	Egg diameter (mm)	Egg volume (mm^3)
FLC	1985	1.27	1.07
	1986	1.23	0.97
CL	1985	1.22	0.95
	1986	1.21	0.93
IL	1986	1.34	1.26

Differences in egg sizes prior to spawning were reflected in variation in the sizes of newly hatched larvae (the age of larvae was verified by comparison with larvae hatched from eggs in the laboratory). Larger newly hatched larvae were found at IL than at FLC in 1985 (unpaired t-test - df_{64}, t=3.39,

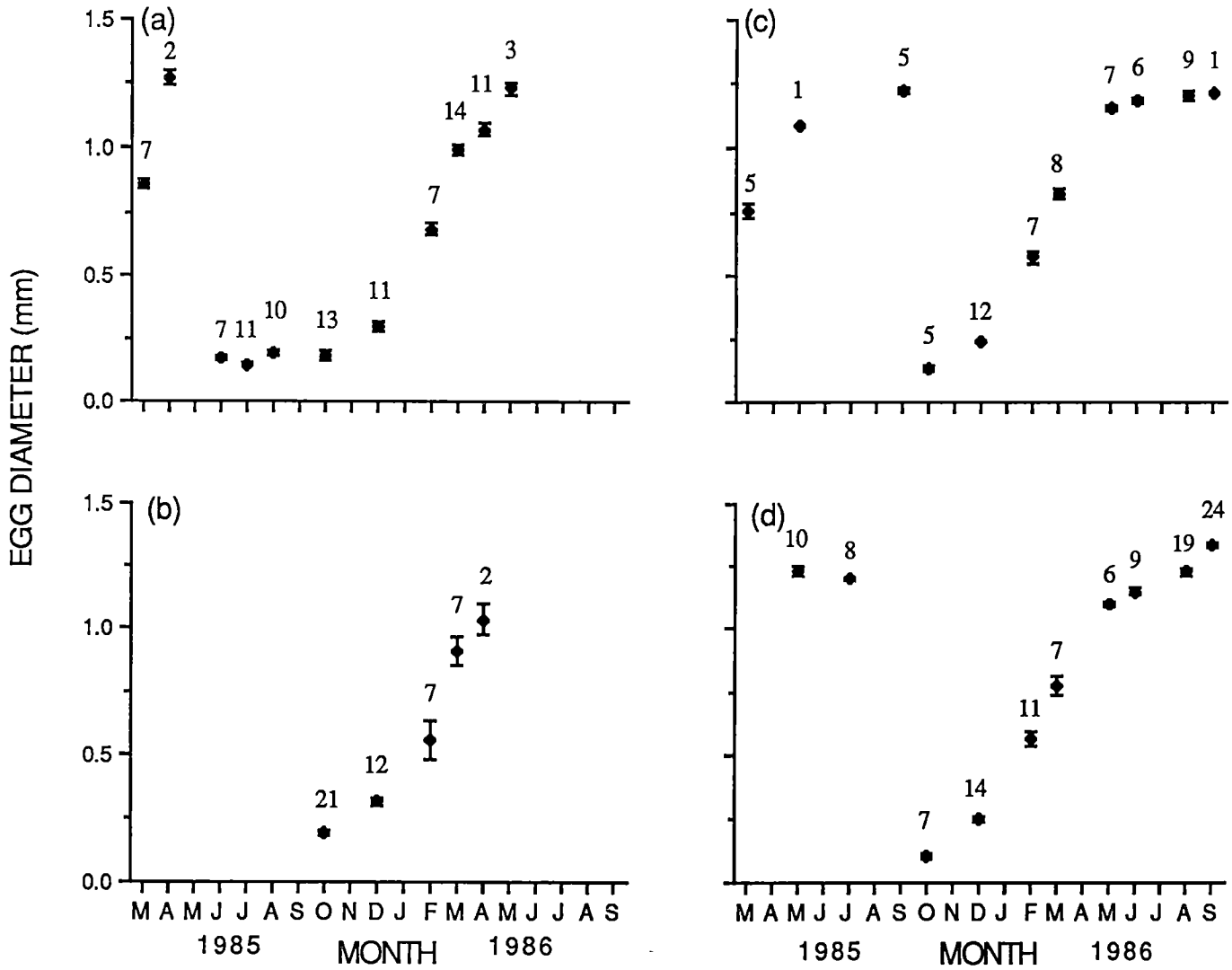


Fig. 3.8 Mean ± SE egg diameters for *G. truttaceus* from (a) FLC, (b) AC, (c) CL and (d) IL from March 1985 to September 1986. Numbers indicate sample sizes of fish.

$p<0.01$) (Fig. 3.9). Although no pre-fertilized eggs were able to be measured for AC females immediately prior to spawning, newly fertilised eggs (eggs which had cleaved no more than four times) collected at AC in May 1986 were significantly smaller than those collected at IL in September 1986 (df_{17}, $t=11.24$, $p<0.001$) (Table 3.9).

The model used to describe variation in fecundity with standard length was $\log_{10}F = \log_{10}a + b \log_{10}L$ where F =fecundity, L =standard length and a and b are constants. The separate and common regression equations for fecundity against length are given in Table 3.10. A comparison of fecundities indicated no differences between localities for 1985 (Fig. 3.10.a). The regression of fecundity against length for CL females in 1986 was significantly different from regressions for FLC and IL females (ANCOVA - CL/FLC: for slopes $df_{34,1}$, $F=5.55$, $P<0.05$; CL/IL: for slopes $df_{50,1}$, $F=6.96$, $p<0.05$), however no difference existed between fecundities of CL and AC females (Fig. 3.10.b).

Where significant correlations occurred for egg diameter with body size and fecundity with body size, these variables were corrected for body size by the use of residuals from regressions of egg diameter and fecundity with standard length. These values were used in plots of fecundity versus egg diameter (Fig. 3.11). It was found that egg diameter and fecundity were not significantly correlated for FLC, CL and IL females in the month prior to spawning.

3.3.6 Timing of Maturation and Spawning

As has been shown in Section 3.3.3 the timing of maturation is similar at all localities, although fish spawned at different times in riverine and lacustrine habitats (Fig. 3.4 and 3.5). Although egg sizes began to increase earlier than was evident from adjusted GSR's, the onset of maturation occurred during the summer equinox. An example of this, showing the maximum and minimum temperatures and photoperiod at CL and adjusted GSR's for CL females, is given in Fig. 3.12.

Hours of daylight were at their peak in December at *c.* 15 h. Creek fish spawned on a decreasing photoperiod and decreasing temperature about 5 mo later. In 1985 a sharp drop in maximum temperature from April to May may have been the cue for spawning at FLC, however, a less dramatic decrease occurred in 1986 (Fig. 2.5). Flows were low over late summer and early autumn in 1986 at FLC and AC (Fig. 3.13), and congregations of fish at the lower extremity of a pool blocked by a log at FLC appeared to be waiting to migrate downstream to spawn when flow increased (see Section 3.3.7). Flow increased about a week into May 1986 and spawning commenced shortly after.

Lake fish spawned in spring, about 4 mo after creek fish. They spawned on an increasing photoperiod and temperature, although temperatures were still low compared to summer levels (Fig. 2.5). Temperature may be the cue for spawning in lake fish since over the 1986 spawning season an increase in the minimum temperature occurred in the first week in September, spawning activity was observed for the first time that year and ripe, part-spent, and spent fish were collected (Table 3.11). A week later the minimum temperature had dropped, no spawning activities were observed, and a larger proportion of part-spent fish were collected than for the previous week ($X^2=10.3$, $p<0.01$). The

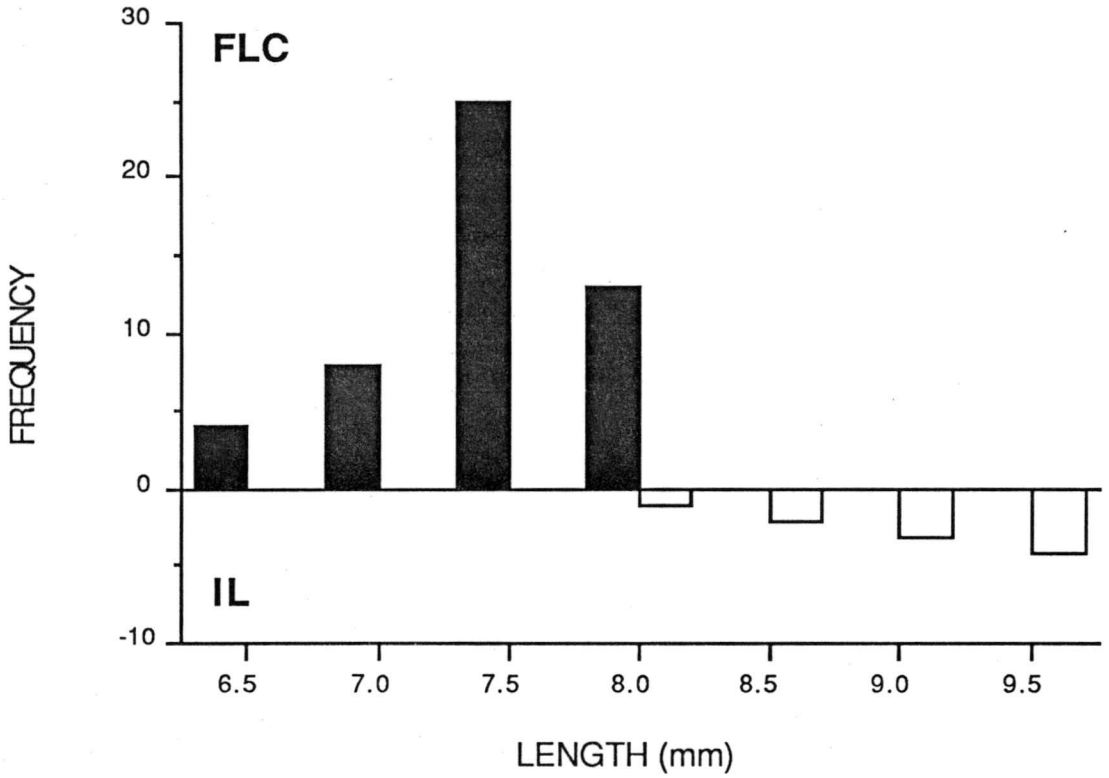


Fig. 3.9 Length / frequency for newly hatched larvae collected from FLC in June 1985 and from IL in October 1985.

Table 3.10 Separate and pooled regression equations for fecundity against standard length for females from FLC, AC, CL and IL, for 1985 and 1986. Equations are calculated from logarithmically transformed data and are of the form F = aL + b; where F is fecundity, SL is standard length and a and b are constants. Allens Creek was not sampled in 1985. Pooled regression for 1986 does not include CL.

Locality	Regression Equation	n	r^2	probabilty
1985				
FLC	F = 3.263 L - 2.867	10	0.81	$p < 0.001$
CL	F = 4.206 L - 4.674	5	0.88	$0.001 < p < 0.01$
IL	F = 2.956 L - 2.185	17	0.83	$p < 0.001$
Pooled	F = 3.362 L - 3.035	32	0.89	$p < 0.001$
1986				
FLC	F = 3.071 L - 2.441	16	0.89	$p < 0.001$
AC	F = 3.609 L - 3.528	8	0.91	$0.001 < p < 0.01$
CL	F = 4.429 L - 5.234	21	0.81	$p < 0.001$
IL	F = 3.335 L - 2.988	32	0.95	$p < 0.001$
Pooled	F = 3.220 L - 2.745	79	0.94	$p < 0.001$

Table 3.11 Percentage of stage V (ripe), part-spent and stage VI (spent) fish at IL during the spawning period in 1986.

Date	Number of fish	Stage V (%)	Part-spent (%)	Stage VI (%)	Maximum Temperature (°C)	Minimum Temperature (°C)
5.viii.1986	32	100	–	–	9	0
3.ix.1986	28	37	16	47	8	2.5
11.ix.1986	31	21	62	17	9	1
19.ix.1986	54	15	21	64	9	3.5
7.x.1986	58	–	–	100	9.5	4.5

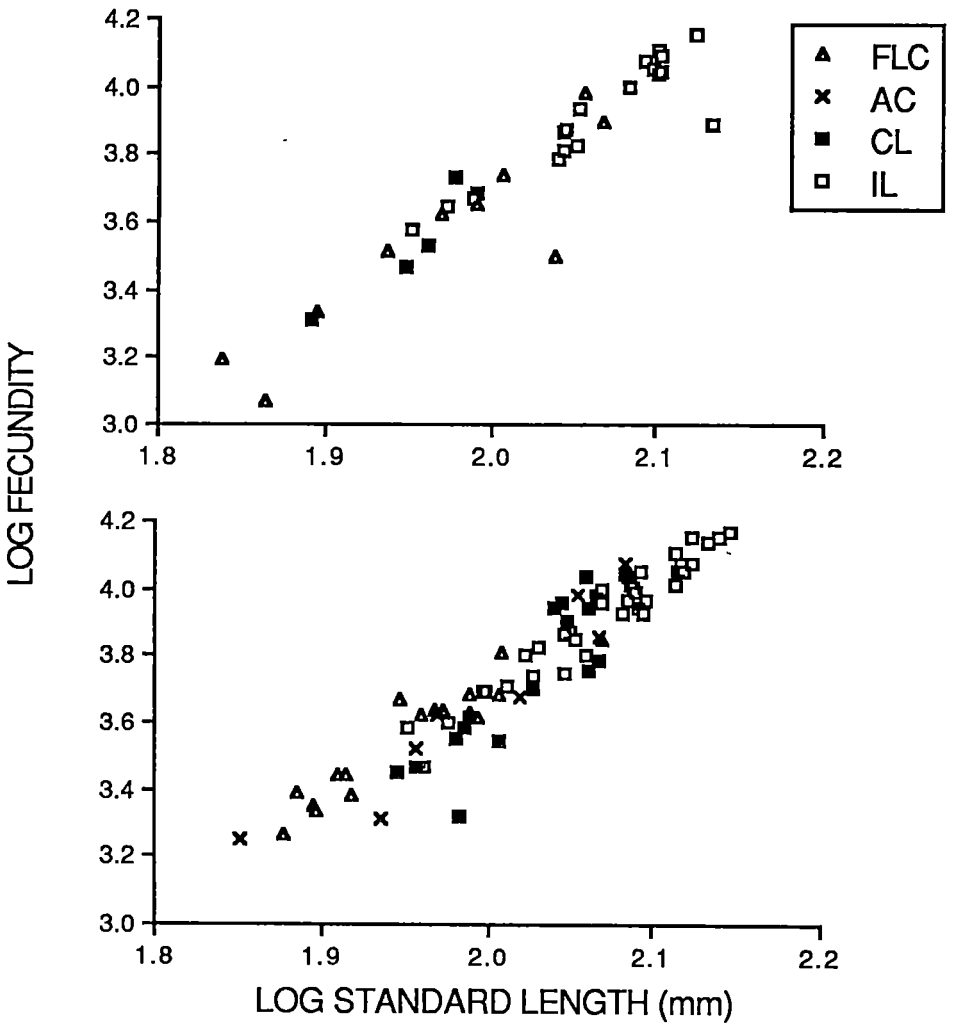


Fig. 3.10 Plots of log standard length versus log fecundity for *G. truttaceus* from FLC, CL and IL in (a) 1985 and from FLC, AC, CL and IL in (b) 1986.

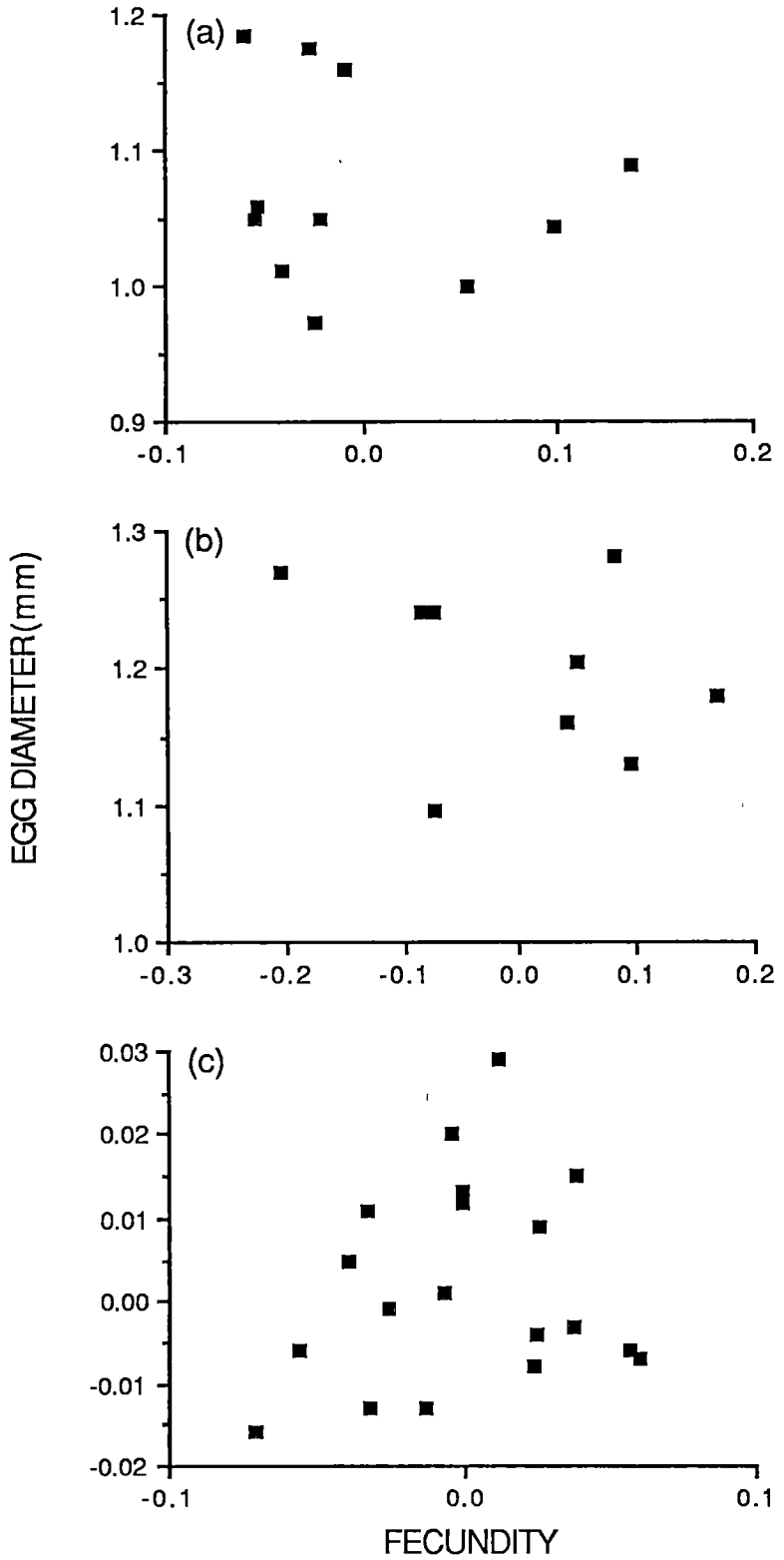


Fig. 3.11 Plots of egg diameter versus fecundity for females from (a) FLC, (b) CL and (c) IL for the month prior to spawning in 1986. For FLC and CL egg diameters are the actual measurements; egg diameters for IL and fecundities for fish from all localities are residuals from regressions of these variables against standard length.

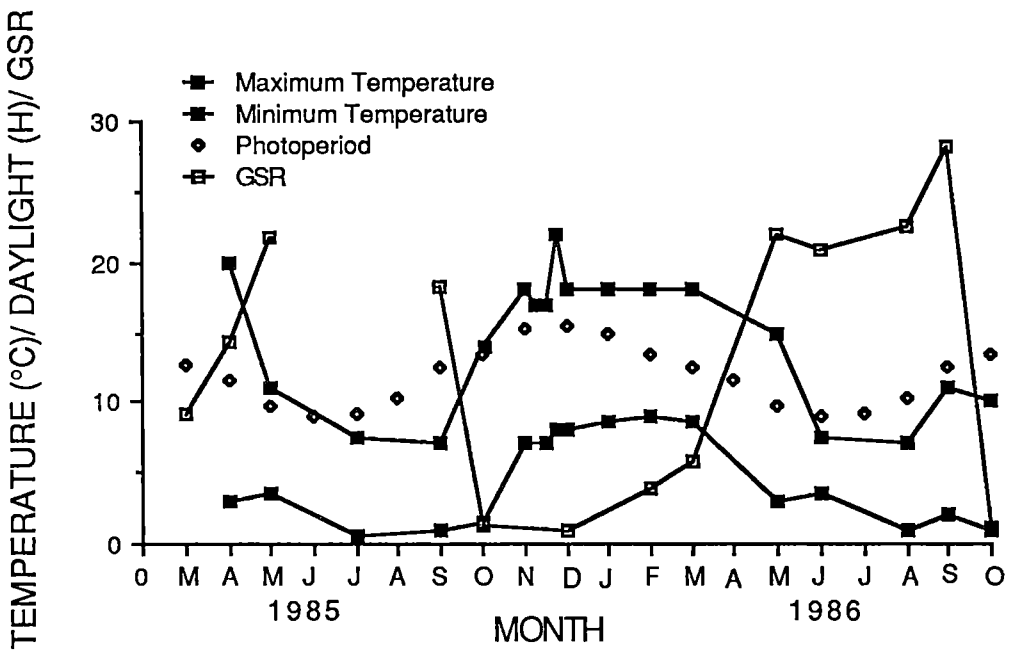


Fig. 3.12 Maximum and minimum water temperatures and photoperiod at CL and adjusted gonadosomatic ratios (GSR) for CL females for the period from March 1985 to October 1986.

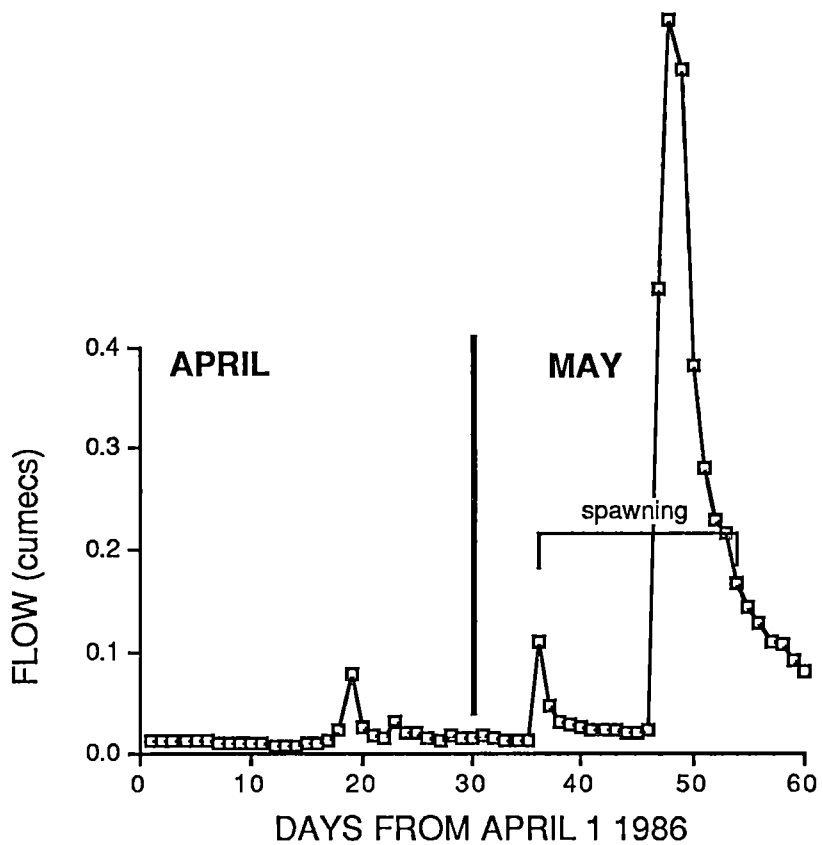


Fig. 3.13 Water flow at AC over the period of April and May 1986.

minimum temperature rose in the next week, spawning activities recommenced and a greater proportion of spent fish were found than for the previous week (X^2=16.3, p<0.001). In the following week maximum and minimum temperatures rose further and all fish collected (n=58) were completely spent. There is some indication that, due to the prolonged presence of part-spent fish, individual *G. truttaceus* may spawn over several days and spawning at particular localities may take place over several weeks.

3.3.7 Spawning

Although the spawning sites of the two lake populations of *G. truttaceus* were located in 1985 and 1986, the exact locations of the deposition of eggs in the creeks were not discovered. However, the approximate area of deposition of eggs was determined for FLC and AC.

Creeks Of four nets placed in FLC on 21 May 1986, net #2 contained 54 newly hatched larvae. No larvae were found in any other nets, so it is presumed that at least these larvae originated from eggs deposited in freshwater, more than 50 m above Fortescue Lagoon. This collection of larvae took place more than four weeks after fish had finished spawning, which indicated an incubation period of at least that period of time.

Autumn 1986 was a relatively dry period and flows were low (Appendix 2). FLC had negligible flow in places and on 16 April 1986 a congregation of fish was observed at the downstream end of a long, shallow, litter-filled pool, barred by a log jam. It was estimated that there were in excess of one hundred fish within this one pool, and all fish examined were either at stage IV (mature) or stage V (ripe). On the same day at AC, external examination of fish collected from the normal sampling site indicated that they had spawned, while downstream some fish caught near the mouth of the stream were at stage V and others at stage VI. It was subsequently discovered by dissection that most fish at the normal sampling site at AC were in fact non-reproductives (stages I and II) and it was presumed that reproductive fish had migrated downstream.

On 25 April 1986 all *G. truttaceus* caught at FLC were at stage V (ripe), however, although the creek was flowing slowly, the log-jam downstream of the congregation of fish had prevented any further migration. All fish collected at FLC on 5 May 1986 were still at stage V; by 15 May 1986 equal numbers of stage V and stage VI fish were found in the previously referred to long pool. An extensive search of the area failed to reveal any eggs. Five days later only a few large fish were found at the normal sampling site and it was presumed that the others had migrated downstream. Most fish along the length of the stream were at stage VI. Eggs collected on this day at AC were between 3 and 7 days post fertilisation (see Section 3.3.8). On 30 May 1986 all fish caught at FLC were spent and eggs collected at AC were between 10 and 12 days old.

Newly hatched larvae were caught in net #2 at FLC on 3 July 1986, and therefore if spawning was complete by 30 May 1986, the eggs had taken at least 34 days to hatch. Eggs and larvae were never caught immediately below normal sampling sites at either creek and the presence of mostly

non-reproductive fish there suggests a downstream migration to spawn. The presence of eggs and larvae in nets above brackish water and above tidal influence suggests that *G. truttaceus* spawns in freshwater.

Lakes All fish collected on 11 September 1985 at IL were spent, and silver gulls, *Larus novaehollandiae*, were seen catching spawning fish near an island in the centre of the lake (Fig. 3.14). Spawning fish were collected by FBA net in this area. Recently fertilised eggs were found amongst the roots of emergent vegetation (*Carex* sp.), below the water surface. Eggs were collected weekly from this time until they hatched and larvae were collected until 10 December 1985.

Other egg deposition sites were located along the northern and north-eastern shores of IL on 25 September 1985. These were also amongst dense clumps of vegetation between 10 and 50 cm below the water surface. On the same day eggs were found at CL amongst clumps of vegetation in small 'alcoves' off the lake (Fig. 3.15). Eggs were only deposited on the lee of the clumps of vegetation, away from severe wave action. Larvae were first collected at IL on 10 October 1985, almost exactly one month after the fish had spawned. The larvae had only recently hatched, as they still had large yolk sacs. More larvae were collected during the following week on 17 October 1985. and were observed swimming close to the surface of the lake. They swam down if disturbed. No larvae were caught at CL, despite extensive use of plankton nets, and it is presumed that they had moved out into the deeper parts of the lake. Fish were again found spawning at IL in 1986 on 3 September at the same sites as the previous year.

3.3.8 Embryonic and Larval Development

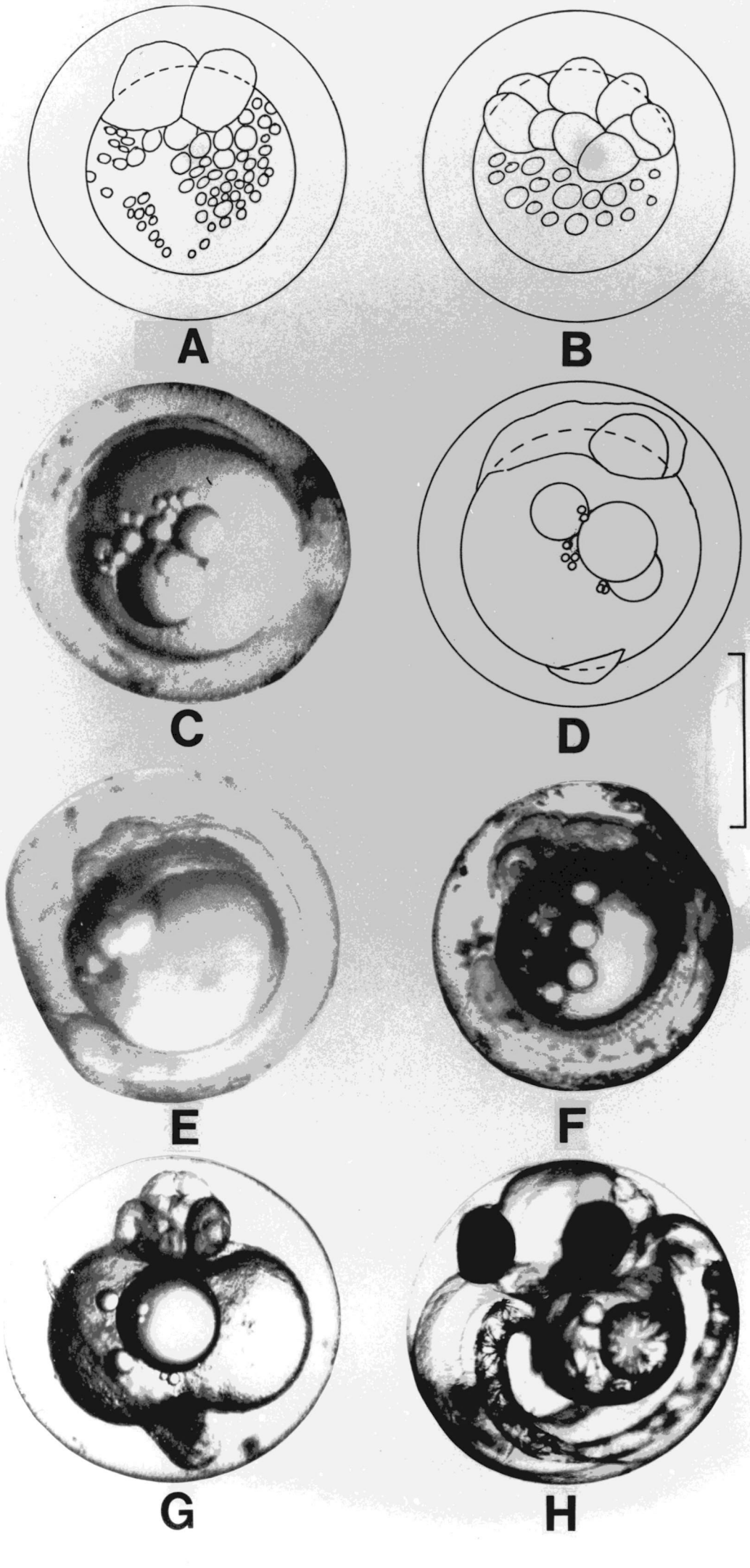
Eggs collected fom IL on 11 September 1985 had been fertilised more than 2 h previously as they had just completed their first cleavage and were at two cell stage (Fig. 3.16a). Benzie (1968b) found that *G. maculatus* eggs maintained at 17° C took *c.* 2 h to reach this stage. The eggs were between 1.6 and 1.9 mm in diameter ($\bar{x}$=1.85), almost perfectly spherical, clear and had a sticky substance which completely covered the egg and allowed adherence to vegetation. A large number of oil droplets were dispersed around the yolk at this stage and the perivitelline space had increased from *c.* 0.1 mm before fertilisation to *c.* 0.3 mm. After 3.5 h the eggs had completed their third cleavage (Fig. 3.16b). After another five hours the apical cap had extended all the way over the yolk, the cephalic and trunk regions of the embryo were just visible (Fig. 3.16c), and the blastopore appeared almost closed. Two large oil droplets and some smaller ones were present at this time. Between 5 and 10 h later the body and head of the embryo had deepened and eyes could be seen, although no lens rudiment was evident (Fig. 3.16d).

About 10 somites became discernable *c.*114 h after fertilisation (Fig. 3.16e). At this stage the eye and lens could be seen clearly and the auditory capsule had developed. The mid- and hind-brain were evident and the embryo extended two thirds of the way around the yolk. The embryo extended almost the whole way around the yolk sac and the somites, eyes and brain had all developed further by *c.*

Fig. 3.14 Photograph of the island in IL where *G. truttaceus* spawned in 1985 and 1986.

Fig. 3.15 Photograph of the alcoves in CL and the vegetation amongst which *G. truttaceus* spawned in 1985 and 1986.

Figs. 3.16.a-h Photographs and line drawings of developing *G. truttaceus* embryos. See text for descriptions. Bar represents 1 mm.


A
B
C
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H

200 h after fertilisation (Fig. 3.16f). About 282 h after fertilisation the somites extended the length of the body, the heart could be seen beating and the embryo was curled more than once around the yolk. The neural fold (Benzie, 1968b) could be seen as could other details of the head region. The eyes began to pigment soon after this last stage (Fig. 3.16g), tail somites were well developed and the notochord could be seen extending to the tip of the tail. The eyes had become more pigmented, neural folds appeared closed and the heart could be seen beating strongly *c.* 360 h after fertilisation. Only two oil droplets, one big and one small, were present and the embryo began moving slightly within the egg. The eyes were densely pigmented after 480 h (Fig. 3.16h), the lower jaw could be seen and melanophores on the skin below the yolk sac and gut were well developed. The embryo twitched frequently and the small pectoral fins were observed for the first time. Approximately 526 h or 24 days after fertilisation the eggs hatched.

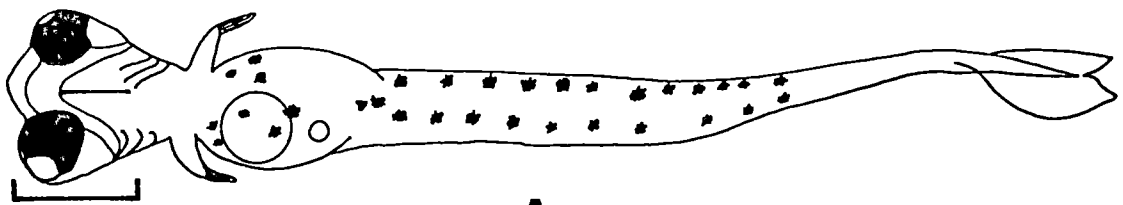
Newly hatched larvae from IL eggs ranged from 7.5-9.0 mm ($\bar{x}$=8.5 mm) in length. Eyes were blue-black with a silvery tinge, the yolk sac was large and measured *c.* 0.9 mm in length. About 20 dendritic melanophores extended the length of the body below the intestine and a similar number along the dorsum (Fig. 3.17a). The auditory capsule, containing an otolith, could be seen and yellow pigment (guanine) was present on the dorsum of the head and body. Muscles of the body wall were well developed and larvae swam vigorously. Details of the head of a newly hatched larvae are shown in Fig. 3.17b. Four days after hatching the yolk sac had reduced, the pectoral fins were functional and the gill slits were obvious. Eleven days later, the oil droplet in the yolk sac, although small, was still present, jaws had developed further and the operculum could be seen (Fig. 3.17c). The oil droplet had disappeared by 20 days after hatching and the caudal fin had formed by constriction between it and the dorsal and anal fin folds. The gill slits were now very obvious and the head, in relation to the body, had reduced in size. The mouth was large and well developed and feeding had commenced, as copepods were seen in the gut of several larvae.

Twenty-five days after hatching, the dorsal and anal fins had formed fully, the rays being fine and hair-like (Fig. 3.17d). The thicker rays of the caudal fin were developing and the pectoral fins had enlarged and were splayed The pelvic fins could not be seen. Sections of the gut could be distinguished and the circulatory system was well developed. It was at this stage that all laboratory reared larvae died and no more wild larvae could be collected.

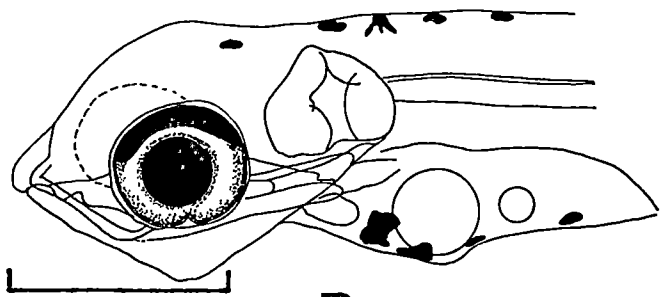
3.3.9 Growth

The young-of-the-year age group could be followed through the seasons at each locality, although larger sample sizes in the creeks allow for a better interpretation of cohorts (Fig. 3.18 to 3.21). Although creek fish hatched at the beginning of winter (June), it was not until the following summer, when they returned from the sea, that they appeared in the population (Figs. 3.18 and 3.19). Lake 0+ fish, having hatched in October, were collected by hand net some 4-6 weeks into their larval life. After this time, they were not caught until late summer, when they appeared in the electrofishing samples in small numbers at first, with catches increasing as time progressed (Figs. 3.20 and 3.21).

Figs. 3.17.a-d Drawings of developing *G. truttaceus* larvae. See text for descriptions. Bars represent 1 mm.



A



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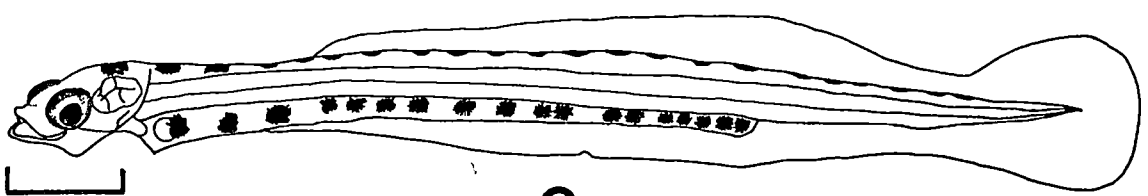
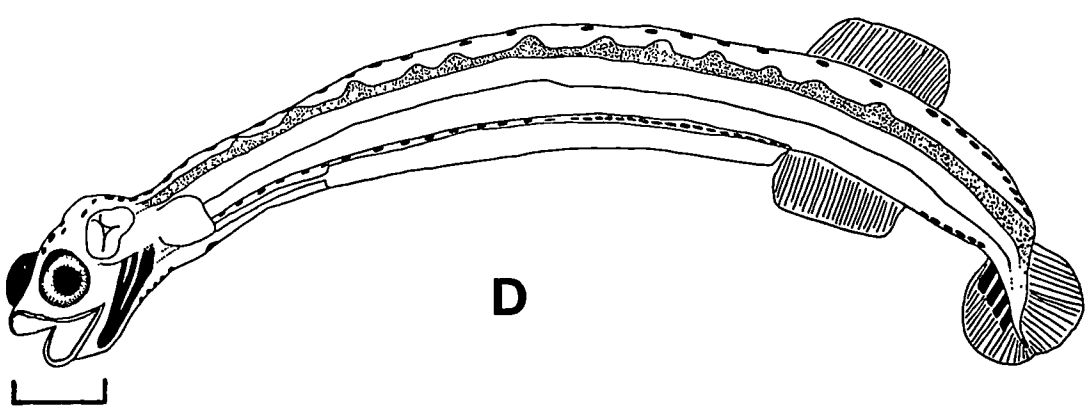


C



D

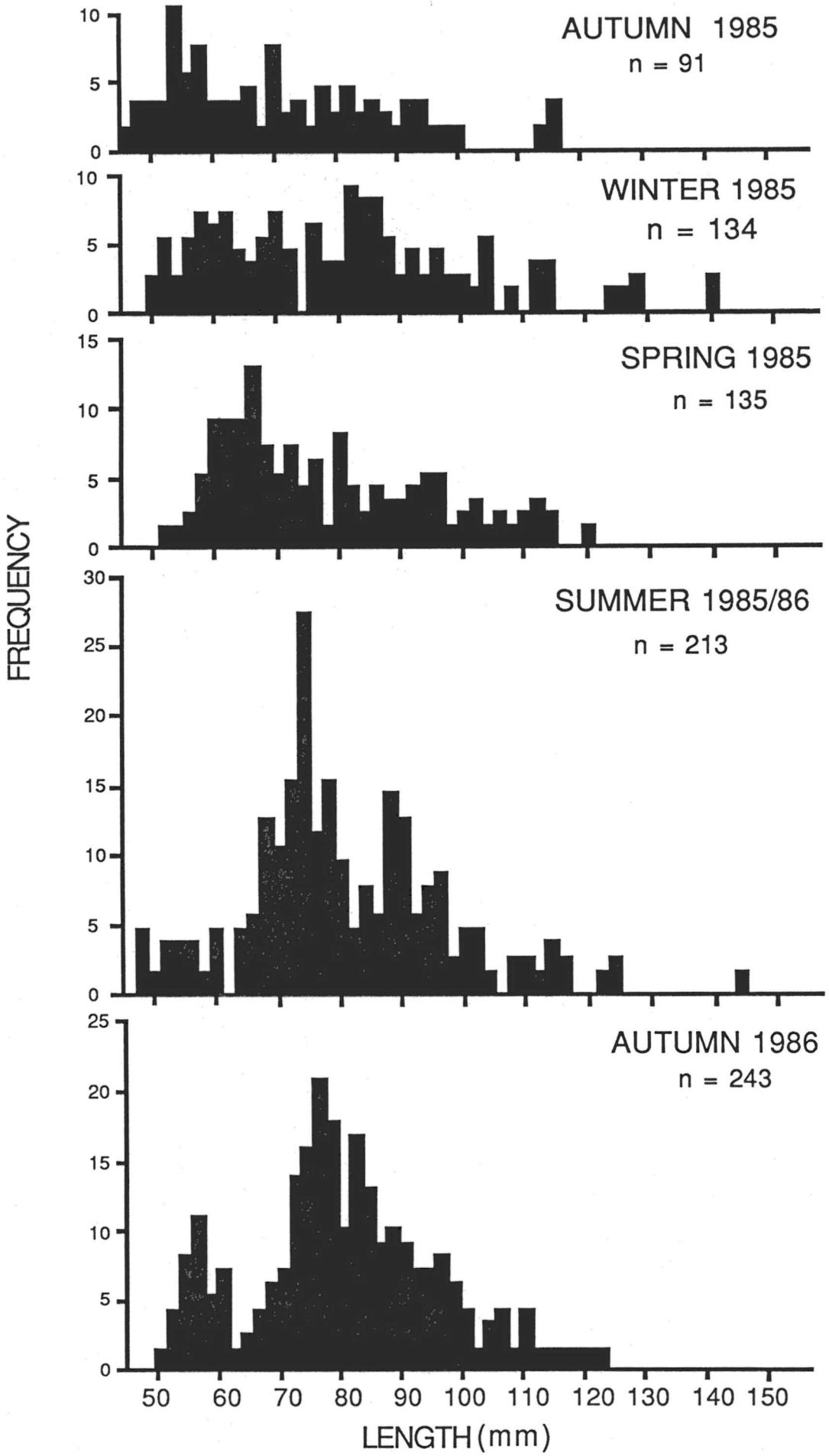


Fig. 3.18 Seasonal length-frequency distributions for pooled sex *G. truttaceus* collected at FLC from Autumn 1985 to Autumn 1986.

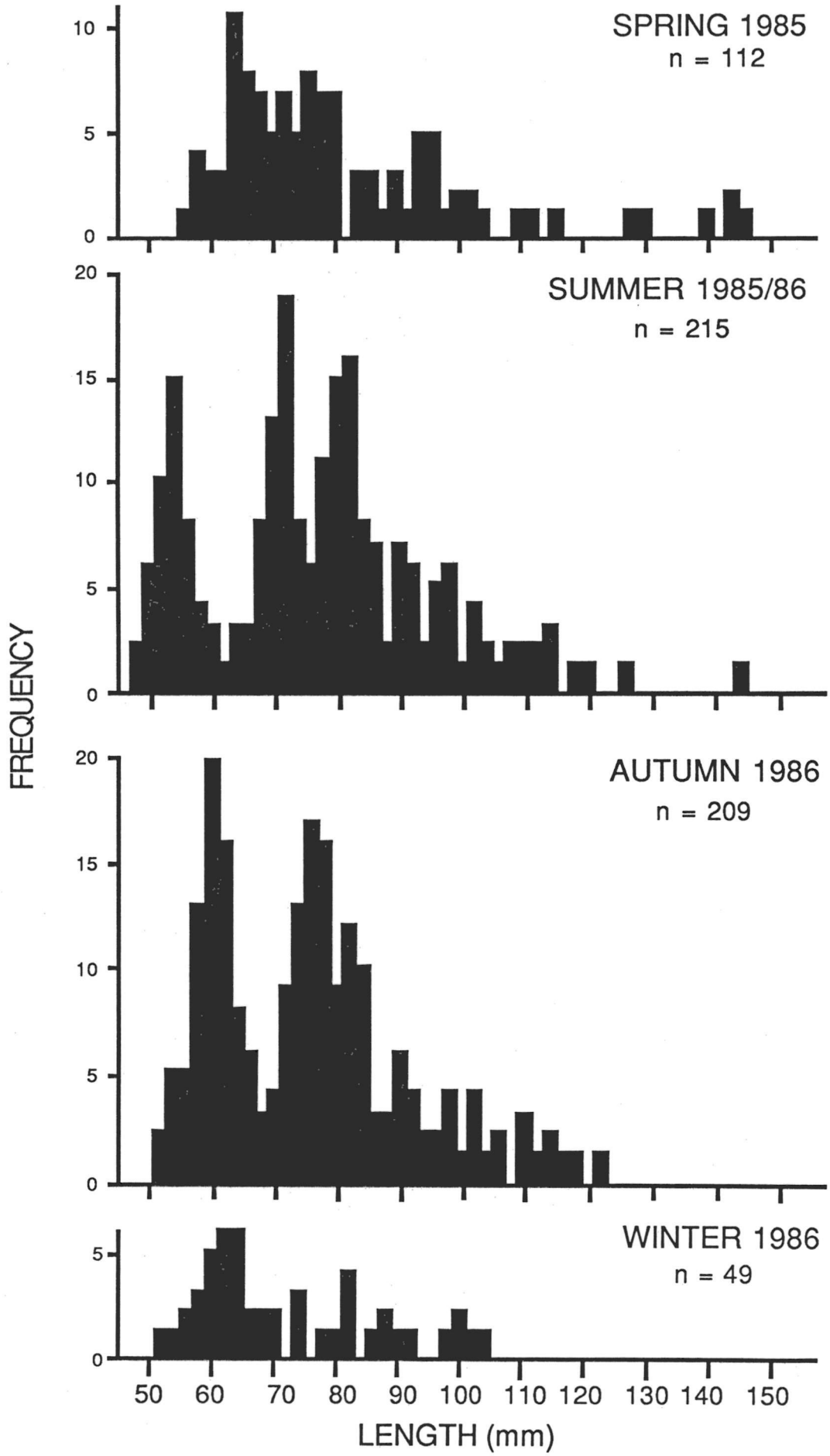


Fig. 3.19 Seasonal length-frequency distributions for pooled sex *G. truttaceus* collected at AC from Spring 1985 to Autumn 1986.

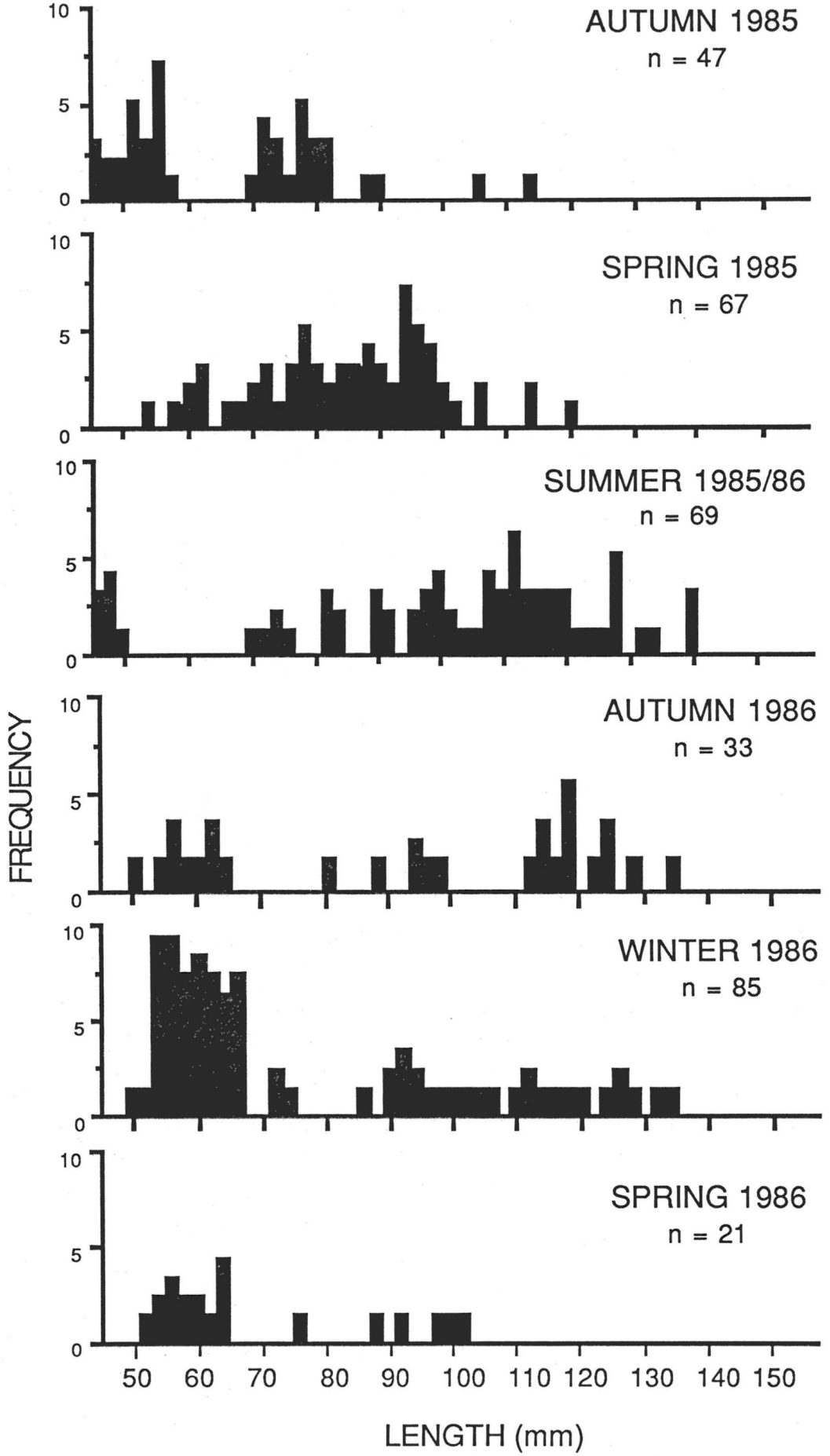


Fig. 3.20 Seasonal length-frequency distributions for pooled sex *G. truttaceus* collected at CL from Autumn 1985 to Spring 1986.

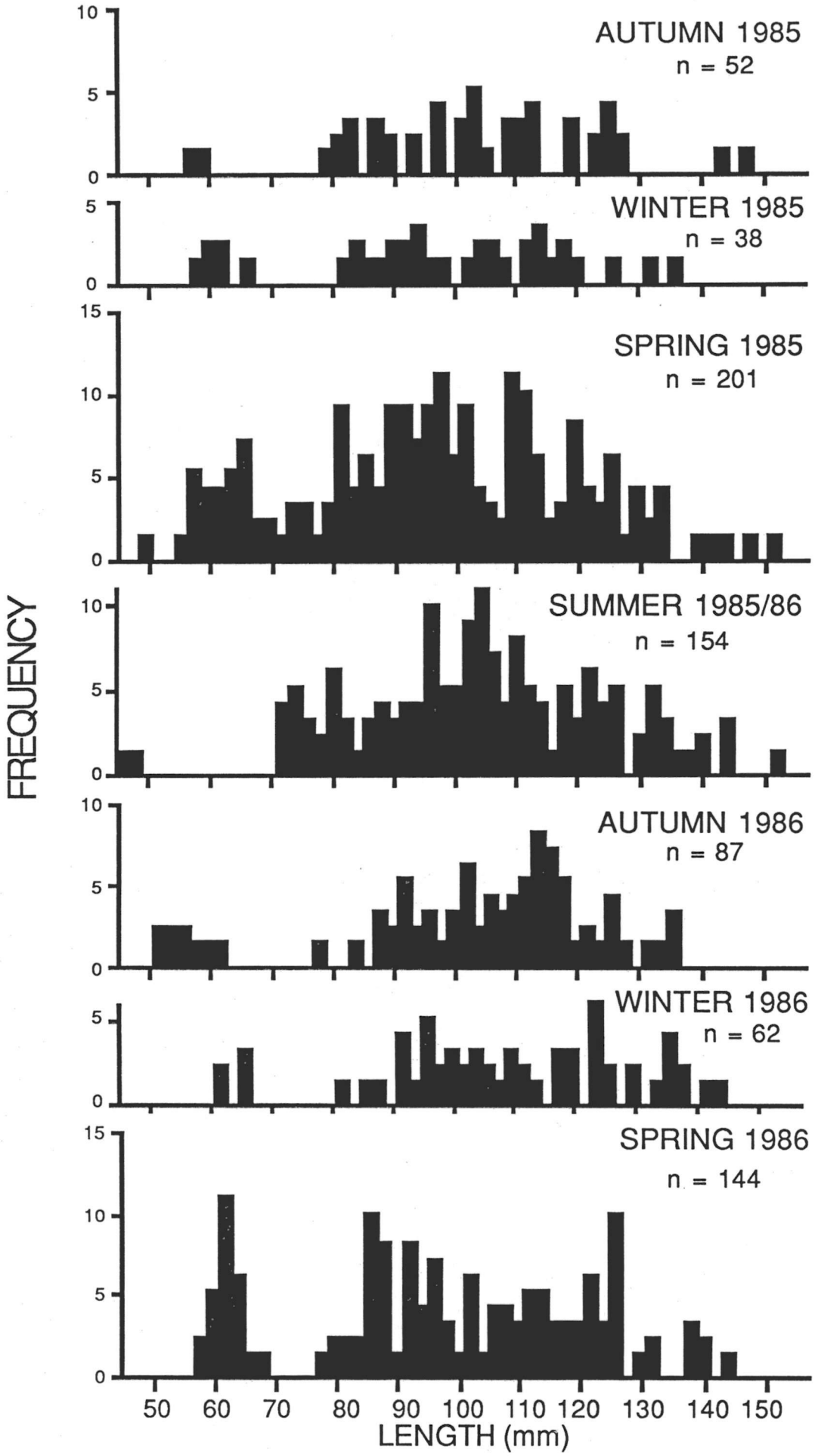


Fig. 3.21 Seasonal length-frequency distributions for pooled sex *G. truttaceus* collected at IL from Autumn 1985 to Spring 1986.

Growth was greatest in all age groups at all localities between spring and autumn (Fig. 3.22). Growth was slowest, and sometimes no growth occurred, between winter and spring. Although the 0+ lake fish were smaller than 0+ creek fish when they first appeared in samples (due to them being *c.* 4 mo younger), the lake fish grew faster, so that by the following winter creek and lake 0+ fish were the same size (Fig. 3.22).

At age 1 fish from riverine and lacustrine populations were of a similar size (Fig. 3.23). By age 2, IL fish were larger than FLC and CL fish (unpaired t-test - IL/FLC: df_{17}, $t=3.68$, $p<0.01$; IL/CL: df_9, $t=2.52$, $p<0.05$), but FLC and CL fish were the same length. By their 3rd birthday fish from both lakes were larger than FLC fish (unpaired t-test - FLC/CL: df_{47}, $t=3.30$, $p<0.01$; FLC/IL: df_{37}, $t=2.64$, $p<0.05$). At age 4 there were no significant differences between fish from any locality, probably due to large size variations.

Walford plots, with each point representing a value for a season, are shown for all four localities in Fig. 3.24. With the exception of FLC fish, there appeared to be relatively constant growth with age, such that the L_∞ for IL fish (405 mm) was much greater than the largest fish measured at this locality (191 mm) and for AC and CL fish the Walford lines actually diverged from the diagonal. Von Bertalanffy growth equations have not, therefore been used as a way of describing growth in *G. truttaceus*.

3.3.10 Field Trips to Other Lakes

A summary of data obtained from collections of fish from Rocky Lagoon, Little Blue Lagoon and Perched Lake is shown in Table 3.12 The fish from Rocky Lagoon, Little Blue Lagoon and Perched Lake were at similar stages of development as their conspecifics in CL and IL, and therefore the synchrony of gonadal maturation appears to extend to other lakes besides CL and IL. Although the three fish from Perched Lake were small in length ($\bar{x}\pm SD = 61.0\pm4.6$), they were all aged by examination of their otoliths and found to be greater than 1+.

3.3.11 Maintenance of Fish for One Year

Of the fish maintained in the laboratory for one year, only one matured and was at Stage IV when preserved. All the rest were in good condition, having large amounts of visceral fat but their gonads were undeveloped. The mature female was from IL had a GSR of 28.8, it was 87.1 mm long and 1+ in age. Mean (±SE) egg diameter was 1.65±0.02 mm and the ovaries contained a total of 1308 eggs. The eggs were larger than any found in females at IL.

3.3.12 Parasites

The dominant parasite of lacustrine *G. truttaceus* was a trematode metacercaria, which occurred as pigmented cysts beneath the skin of the fish host. The cysts were similar to *Diplostomum galaxiae* Smith and Hickman (although the exact taxanomic status was not determined), found in *Galaxias auratus* from Lake Crescent on the lower Central Plateau of Tasmania (Smith and Hickman,

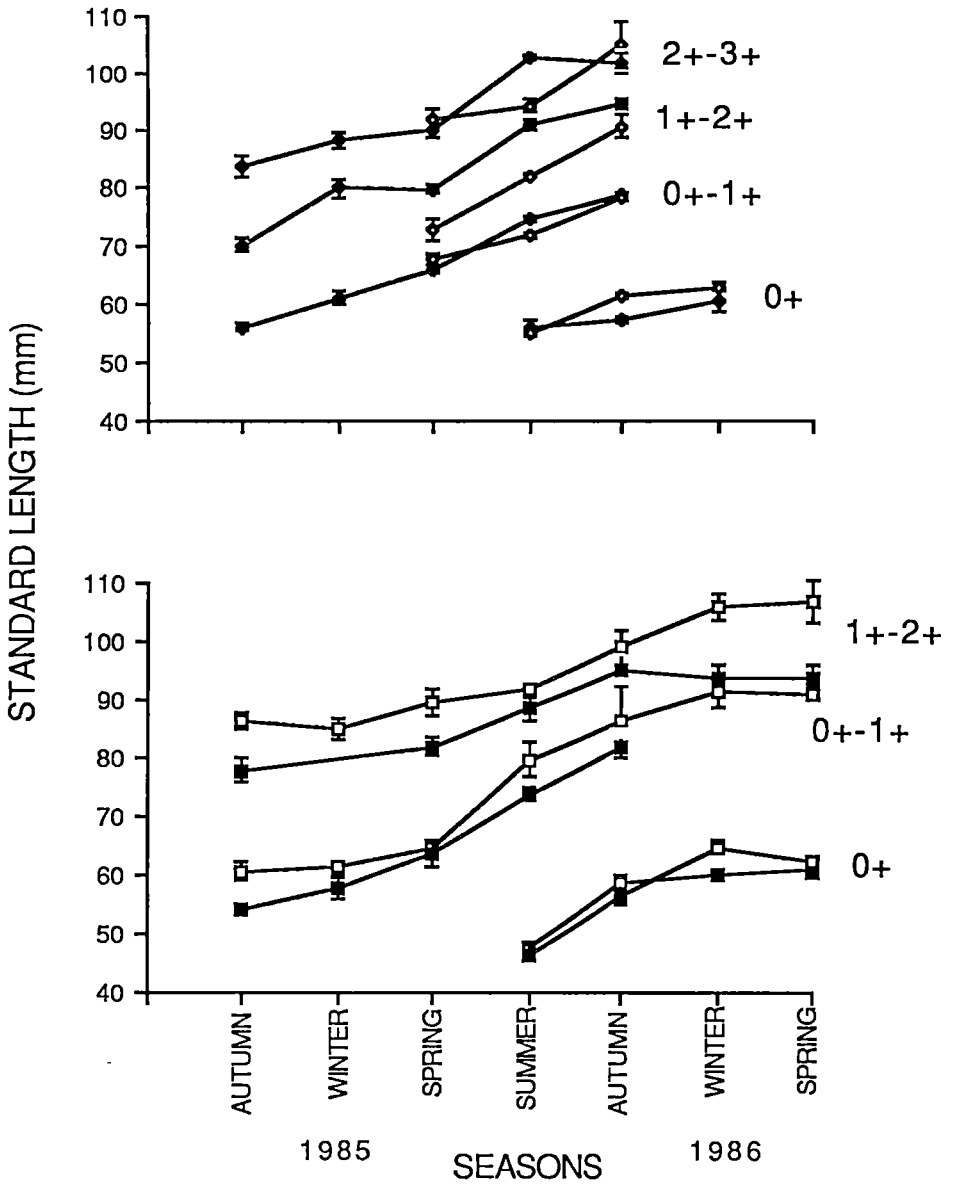


Fig. 3.22 Seasonal growth in mean length ± SE of age-classes for pooled sex fish from FLC (closed diamonds), AC (open diamonds), CL (closed squares) and IL (open squares) during the period Autumn 1985 to Spring 1986. Numbers to the right of graph indicate age groups.

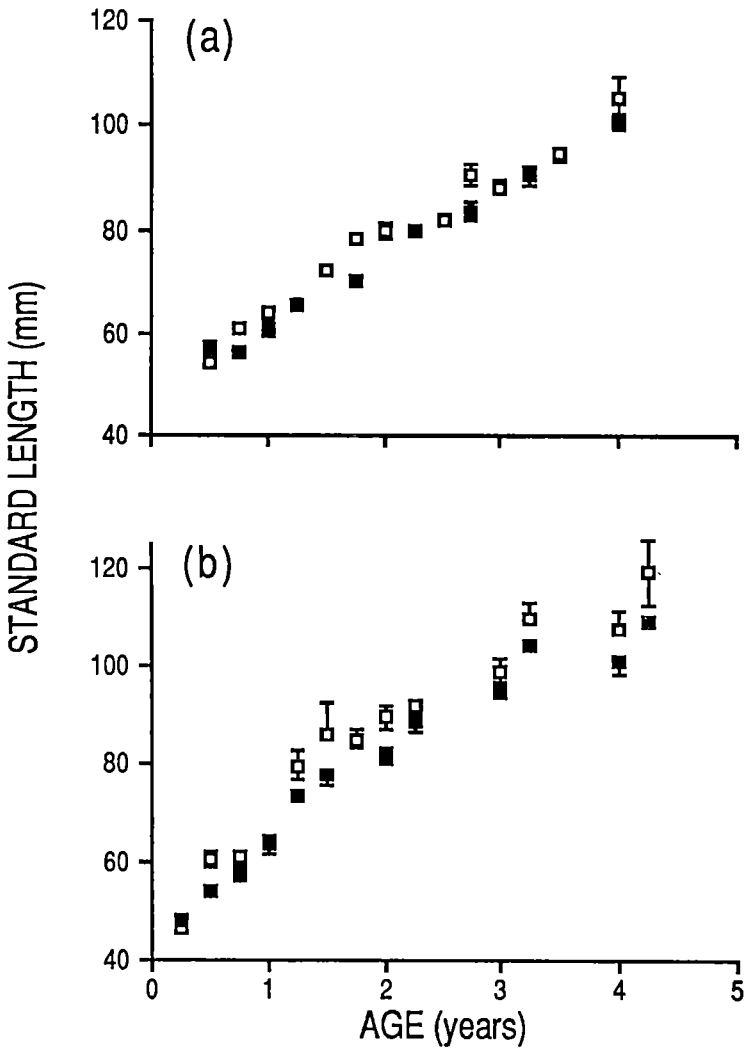


Fig. 3.23 Mean length ± SE with age for pooled sex fish from (a) creeks (FLC-closed squares; AC-open squares) and (b) lakes (CL-closed squares; IL-open squares).

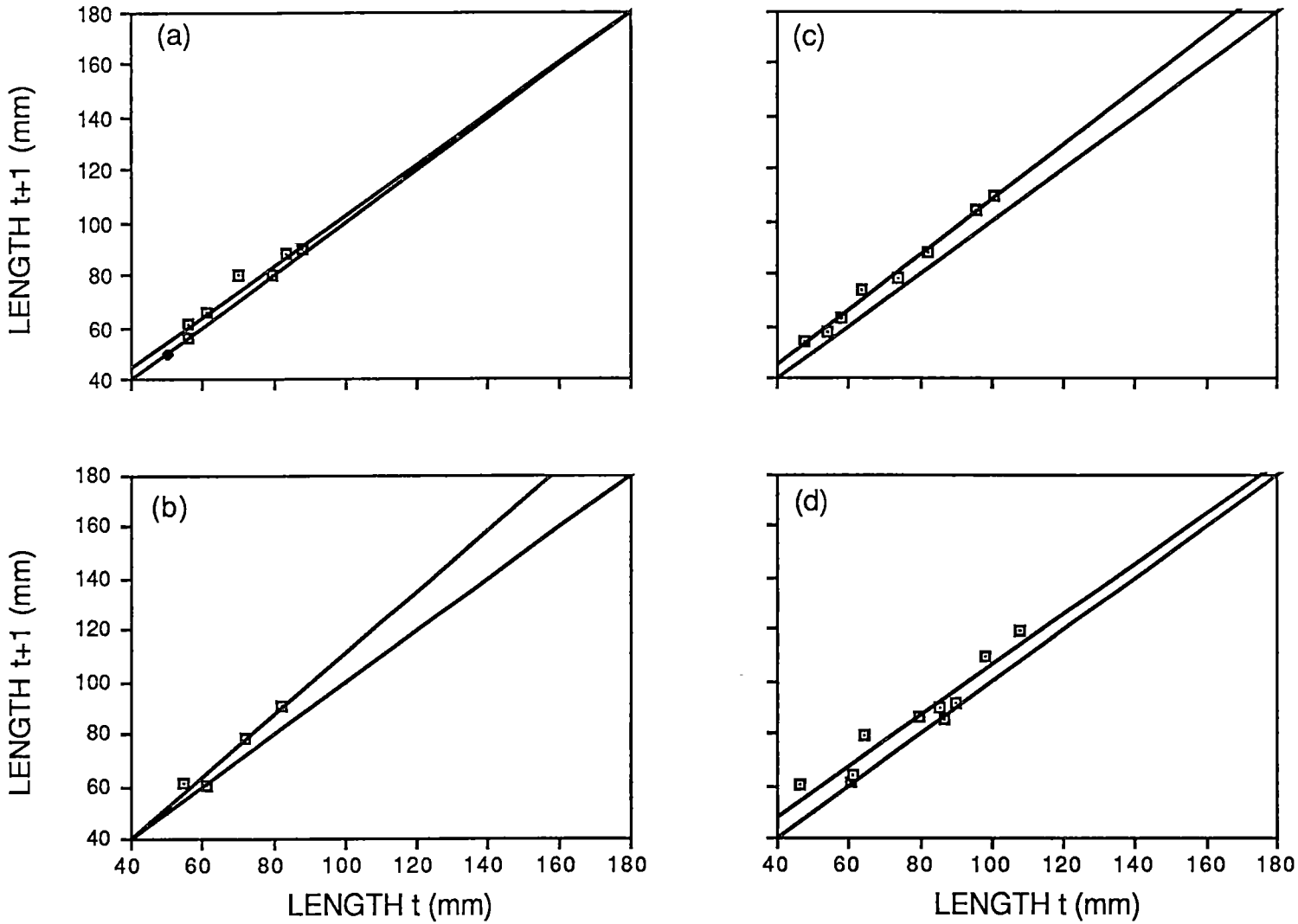


Fig. 3.24 Walford plots of length at age t + 1 against length at age t for pooled sex fish from (a) FLC, (b) AC, (c) CL and (d) IL. Fish are separated into seasons.

Table 3.12 Numbers, lengths, weights, gonadosomatic ratios (GSR) and maturity stages for fish from collecting trips made to Rocky Lagoon, Little Blue Lagoon and Perched Lake. Values are means ± SD.

Locality	Number of fish		Length (mm)	Weight (mm)	GSR		Stage
	female	male			female	male	
Rocky Lagoon	1	7	92.4±16.0	14.5±8.5	15.7	17.7±2.5	IV
Little Blue Lagoon	5	2	90.3±7.3	10.1±3.3	0.87±0.39	0.64±0.34	III
Perched Lake	3	--	61.0±4.6	3.1±1.0	2.69±0.38	--	III

1983). Riverine fish were not infected with the same parasite, but had another trematode metacercaria within the body cavity. This was commonly embedded in the liver or in connective tissue adjacent to the intestine. This parasite appeared to be similar to *Apatemon gracilis* (Rudolphi), also found in the body cavity of *G. auratus* (Smith and Hickman, 1983). Occasionally nematodes were found in large numbers in the swim bladder of fish from both habitat types. Adult cestodes were found only in lacustrine fish and were often found attached to the stomach or embedded in the gonad. The relative numbers of parasites increased in number with increasing fish size (Fig. 3.25), and FLC and IL fish appeared to possess greater numbers of parasites than fish from AC and CL.

3.4 DISCUSSION

The variable nature of habitats can influence life history traits of animals even though populations may be geographically quite close. Traits providing an advantage in one environment may be of no use or even detrimental to the survival of individuals in another. Physical and chemical parameters, food availability, predation and other factors can cause variation in life history traits, and the expression of these traits may be purely environmentally induced and have no genetic basis. On the other hand traits initially providing an advantage in a particular environment may be selected for and so persist through subsequent generations if heritabilities of these traits are high. It is important when looking at life histories, that variation within populations, especially between sexes, is not overlooked. Interpopulation or intraspecific life history variation was found in *G. truttaceus* in the present study both between habitat types (streams and lakes) and within habitat types, although differences were more pronounced in the former.

Many species of fish have distinct secondary sexual characters by which the sexes can be distinguished. These can range from bright colours in males, to nuptial tubercules, to specialised intromittent organs involved in reproduction. Such organs are well known in the class Chondrichthyes where males of many species possess clasping organs and in live bearing poeciliids where males have gonapodia. Several Australian fish species are sexually dimorphic in the shape and position of genital papillae, such as *Gobiomorphus coxii, Gobiomorphus australis* (Hoese *et.al.*, 1980) and the Tasmanian whitebait, *Lovettia sealii* (McDowall, 1980).

The occurrence of sexual dimorphism based on secondary sexual characters in galaxiids is rare and has been thought to be restricted only to *Galaxiella pusilla* (and possibly *G. munda* and *G. nigrostriata*), where the male is smaller than the female and has a distinct red stripe down its flanks (McDowall, 1978a). The existence of distinctive genital papillae has not been recorded in galaxiids before and their functions, if any, are not known. During the present study these papillae have been observed in *Galaxias auratus* and *G. brevipinnis* as well as *G. truttaceus* .

When the total number of fish from each site was considered, sex ratios of *G. truttaceus* at the four localities were essentially similar. In all cases females were more numerous than males. The sex

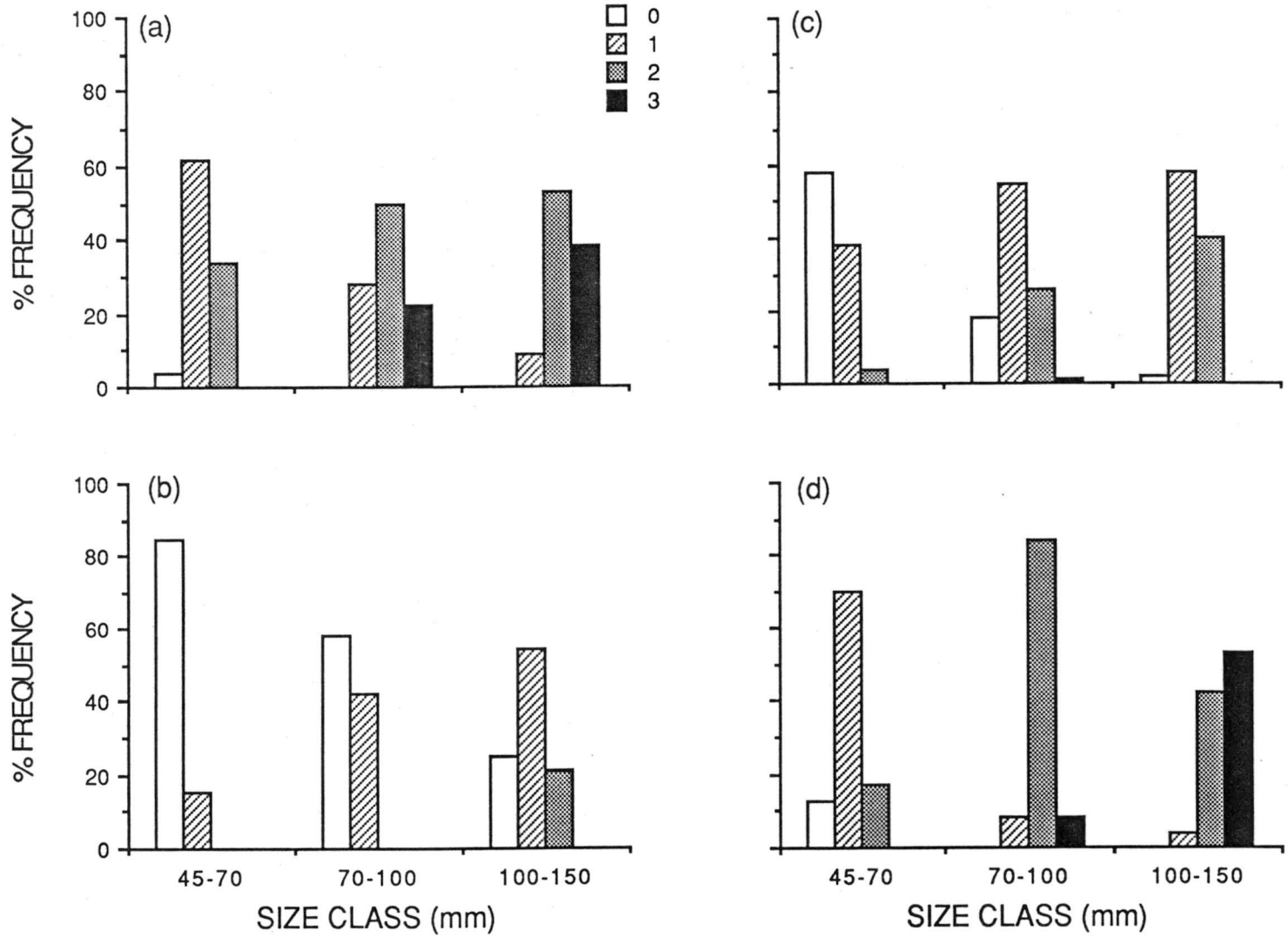


Fig. 3.25 Percentage frequency of fish from (a) FLC, (b) AC, (c) Cl and (d) IL of different size-classes possessing (0) no parasites, (1) 1 - 10 parasites, (2) 10 - 50 parasites and (3) >50 parasites.

ratios of different size classes for creek populations indicated some significant differences which were always in favour of females. A similar situation was found for lake populations, except for the 65-85 mm size class. In this group males outnumbered females and the reason for this is uncertain. Differences in proportions of sexes at spawning times may be due to differential spawning behaviour, which has also been suggested for *G. vulgaris* (Benzie, 1968a, *G. fasciatus* (Hopkins, 1979b), *Paragalaxias dissimilis* and *P. eleotroides* (Fulton, 1982). In general, populations of galaxiid species have not shown overall differences in proportions of sexes (Benzie, 1968a; Hopkins, 1971, 1979a; Cadwallader, 1976; Eldon, 1979b; Fulton, 1982; Humphries, 1986).

The average reproductive female *G. truttaceus* was larger than the average reproductive male *G. truttaceus* at all localities. This is thought to partly reflect the more rapid growth of females and partly their greater longevity which has also been shown to occur in *G. fasciatus* (Hopkins, 1979b), *G. divergens* (Hopkins, 1971) and *G. maculatus* (Pollard, 1971a) females. Size differences between populations can be attributed to different growth rates and, at IL, a larger proportion of older fish. *G. truttaceus* generally matured at age 2 and at this age IL fish of both sexes were significantly larger than fish at FLC and AC. Some male *G. truttaceus* from CL and IL matured in their first year of life, as do *G. fasciatus* (Hopkins, 1979b) and *G. vulgaris* (Cadwallader, 1976), where it is thought that maturity is dependent upon size. Reproductive 0+ *G. truttaceus* males were found to be no larger than non-reproductive males. Some galaxiids such as *Galaxiella pusilla, Neochanna burrowsius, P. dissimilis* and *P. eleotroides* mature and spawn in their first year and are in general small, shorter-lived species (Humphries, 1986; Eldon, 1979b; Fulton, 1982), while others such as *Galaxias fasciatus* and *N. apoda* do not spawn until age 2 or older (Hopkins, 1979a, b; Eldon, 1978).

Gonadal development followed a similar pattern for all populations of *G. truttaceus*. Maturation commenced at the beginning of summer and mature fish were present before the onset of winter. Lake reproductive males did not develop further once fish in the creeks had spawned, however, reproductive lake females did continue to develop more gonad until spawning in September, some four months later. Relative to body size, lake females invested significantly more in reproduction than creek females at spawning, whilst there were no differences between males from any locality, although relative to body size IL males had invested significantly more gonad than CL males two months previously. As CL females aged they invested proportionally more in reproduction, as shown from the relationship between gonad weight and body weight. However, the change in reproductive investment for females from FLC and IL was not as dramatic. At age 2 FLC females and IL females had similar reproductive investment but double that of CL females; at age 3 reproductive investment of FLC females had decreased while reproductive investment of CL and IL females had increased. By age 4, CL females had a greater reproductive investment than FLC females, and IL females had a greater reproductive investment than females at FLC. The implications for larger reproductive investment will be discussed further in Chapter 5.

In the study of fish, reproductive investment has usually been expressed as the ratio of gonad

weight to body weight or GSR and, although the inadequacies of this measure have been made apparent (de Vlaming, *et al.*, 1982), this is the only estimate of reproductive investment used by other workers studying galaxiids. In fish, females generally invest more in reproduction than males, however, this was not found to be the case for creek *G. truttaceus*, where similar gonad weight/body weight relationships were found for stage V fish of both sexes. Classical GSR's have been calculated for *G. truttaceus* for a comparison with other galaxiid species. Overall stage V female *G. truttaceus* had GSR's between 5 and 36, with a mean of 22.6 and stage V male *G. truttaceus* had GSR's between 6 and 24, with a mean of 17.4. The male GSR is higher than for most other galaxiids and is high compared with many other fish species. This phenomenon may be explained by the skewed sex ratios found at all localities, especially at spawning time where one male may fertilise the eggs of more than one female. The female GSR is comparable to other female galaxiids, although some larger and older *G. truttaceus* females possessed GSR's in excess of 35. At spawning, male and female *Galaxiella pusilla* had GSR's of 9.8 and 16.4 respectively (Humphries, 1986), *Galaxias maculatus* had GSR's of 10.4 for males and 16.5 for females (Pollard, 1971a), *G. fasciatus* had GSR's of 9.6 for males and 19.8 for females (Hopkins, 1979b), *G. vulgaris* had GSR's for male and females of 11 and 23 respectively (Cadwallader, 1976) and *N. apoda* males had GSR's of 9 and females GSR's between 16-26 (Eldon, 1978).

Further comparisons of reproductive investment between species may lead to false conclusions because none of the above authors has considered the effect of body size on reproductive investment. Spurious results can be obtained should there be differences in body size distributions between populations (de Vlaming *et al.*, 1982; Samson and Werk, 1986) and therefore it is preferable to examine the gonad weight body weight regressions. In the present study preliminary investigations comparing classical GSR's and gonad weight/body weight regressions came to different conclusions.

The larger reproductive investment seen for IL females can probably be explained by the fact that at spawning their eggs were significantly larger than those of females from the other populations. In 1986 the eggs of IL females were 9% larger in diameter and 30% larger by volume than those of FLC females. This difference in size appears to be translated to newly hatched larvae and possibly to fertilised eggs. Egg sizes of CL females were not larger than those of FLC females, so the different reproductive investment seen for females at this locality cannot be explained in the same way. From the four populations of *G. truttaceus* egg diameter ranged from 1.21 to 1.34 mm prior to spawning. Galaxiid egg diameters range from *c.* 0.75 mm in *G. gracilis* (McDowall, 1970) to more than 2.5 mm in *N. apoda* (Eldon, 1978), and the pattern relating size of eggs and number of eggs to a particular life history strategy is discussed further in Chapter 5.

Although there were no differences between localities for egg numbers in 1985, the small sample sizes for this year (5 for CL) may have concealed what became apparent in 1986. The slope of the regression of fecundity with length for CL fish in 1986 was significantly greater from that of FLC and IL fish, although no difference existed between regressions for CL fish and AC fish. Therefore it

is postulated that the different gonad weight to body weight regression shown for CL reflects the nature of the change of fecundity with body size.

There is a large variation in egg number within the Galaxiidae, the implications of which will be discussed in Chapter 5. It is sufficient at this stage to note that fecundity in galaxiids varies from between 100 and 300 eggs as for *Galaxiella pusilla* (Humphries, 1986), to as many as 14,000 eggs for a large female *Galaxias maculatus* (McDowall, 1970). Non-significant correlations found between egg size and fecundity for *G. truttaceus* found in the present study imply that these two parameters are acting independently of one another. This appears to be inconsistent with ideas that there are trade-offs between life history traits such as egg number and egg size (see Trendall, 1983)

Although maintenance of *G. truttaceus* from FLC and IL for one year in the laboratory proved unsuccessful in as much as only one fish matured, the results from that one fish indicated a degree of plasticity in egg size and number. The mean egg size and number were both beyond the range for fish from IL of the same length, however, the fact that no other fish matured may indicate the inadequacies of the experimental technique.

In summary, for reproductive fish, lake females invested more, in terms of weight of gonad relative to weight of the body, in reproduction than did creek females, whilst males from all localities invested a similar amount relative to body size. The greater investment of lake females is consistent with the observations that IL females have larger eggs and CL females demonstrate a greater slope on the fecundity versus body length relationship. Due to indications that life history traits can be affected by maintenance of fish in the laboratory, it is possible that many of the differences seen in fish between localities are simply responses to varying environments. However, CL was the only locality where adult and juvenile *G. truttaceus* were presumed to be subjected to a high predation pressure due to the presence of large numbers of brown trout, *Salmo trutta*. In Lake Crescent on the lower Central Plateau of Tasmania, *G. auratus* were also predated heavily by trout to the extent whereby 99% of the stomach contents of 29 trout were *G. auratus* and in 27 of the fish the only food item in their stomachs was *G. auratus* (S. Chilcott, pers. comm.). Although the trout have only been present in the CL for approximately 50-60 years (Dr R. Sloane, pers. comm.) and therefore only 25-30 generations have passed in the lives of *G. truttaceus* since the introduction of trout, producing larger numbers of eggs may be an adaptation to ensure the survival of at least part of a batch of offspring. A genetic basis for the differences in egg number could only be determined conclusively by transplantation experiments, or by controlled breeding in the laboratory over at least two generations.

Some of the differences in life history traits between populations of *G. truttaceus* may simply be attributable to the shift in spawning time from before winter in creek fish, to after winter in lake fish. This extra time allows lake females to develop more gonad, whether it be in the form of larger eggs or greater numbers of eggs. Riverine *G. truttaceus,* like most other diadromous galaxiids, spawns on a decreasing photoperiod and temperature. As has been noted previously, the initial stages of gonadal maturation at all localities were very similar (commencing in December), however, lacustrine *G. truttaceus* delayed spawning for about four months over winter. The gonads of females of the

totally freshwater species *G. vulgaris* began developing between December and February, although males did not begin maturing until autumn, and fish did not spawn until July -September (Cadwallader, 1976). Nevertheless both male and female *G. vulgaris* were mature before winter and maintained that condition until spawning. One totally freshwater species, *P. dissimilis,* does not mature until spring and spawns in summer (Fulton, 1982).

The study most applicable to the present one is that of *G. maculatus* in Lake Modewarre, Victoria, where Pollard (1971a) found that gonad maturation commenced in early autumn (March) and was almost complete in early winter. Final maturation occurred in late winter (August) when fish moved up into spawning streams. The same species in streams in New Zealand began developing in early summer (December) and spawned in late autumn (McDowall, 1968). Pollard (1971a), however, trivialised the differences, suggesting a similar gonadal cycle took place between both forms of *G. maculatus* and that the cycle of lacustrine fish was simply out of step with the diadromous cycle by three months. While the differences in spawning times between Victorian landlocked and New Zealand diadromous populations of *G. maculatus* are similar to those shown by *G. truttaceus*, gonadal maturation commences later in landlocked *G. maculatus*.

The stimulus for the commencement of maturation in *G. maculatus* and *G. vulgaris* is thought to be decreasing photoperiod at lower temperatures (Pollard, 1971a; Cadwallader, 1976), and from the evidence this also appears to be the case for *G. truttaceus*. However, the fundamental difference between riverine and lacustrine *G. truttaceus* is in the spawning time and concomitantly the cue for spawning and not timing of maturation. Benzie (1968a) noticed a temperature difference along the length of the stream in which she was studying *G. vulgaris* and that fish in warmer water spawned before those at cooler temperatures. Those fish dependent upon flooding for spawning are thought to be stimulated to spawn by high flows (Pollard, 1971a; Hopkins, 1979a). In contrast with Benzie's (1968a) observations spawning at AC was thought to have commenced earlier than at the warmer FLC. Low stream flow also seemed to prevent downstream migration of fish at FLC and it was not until higher flows occurred that FLC fish spawned.

The reason why lake fish did not spawn before winter may have simply been a reaction to temperatures which were decreasing too quickly. Minimum temperatures in the creeks decreased slowly over the spring period, hovering between 7.5° and 10° C for several weeks, while minimum temperatures over this time in lakes fell from about 7.5° to 2.5° C (see Section 2.2.5). *G. truttaceus* larvae, hatching in late spring, had a plentiful food supply of zooplankton and therefore, as workers on other lacustrine galaxiids have suggested, no matter what the reason for the shift in spawning time, the conditions for larvae are better in spring and summer for food and temperature reasons (Pollard, 1971a; Fulton, 1982).

From data obtained by sampling other lakes besides CL and IL, it appears that the two additional highland lake populations and one lowland lake population were in synchrony in maturation schedules with the four main populations of *G. truttaceus*. Specimens of *G. truttaceus* collected at Bronte Lagoon in early autumn (March) by Andrews (1976) were thought to be immature but from

the results of the present study, it appears likely that these fish were at a low stage of development, but were nevertheless maturing. *G. maculatus* collected in April from Bouler's Lagoon in north-east Tasmania were only partly developed, suggesting a later maturation than seen for either creek or lake *G. truttaceus* (Andrews, 1982).

G. truttaceus spawned in freshwater in both streams sampled. Nets placed in FLC for a day each week over two successive breeding seasons and at AC over one breeding season demonstrated that the fish spawned above brackish water, but below the normal adult habitat. Some downstream migration occurred, as described for other diadromous galaxiids, however, the suggestion that this species spawns in brackish water or the sea (Scott, 1941) is not substantiated. No definite migration occurred in either lake, although fish did move a short distance from the normal habitat. Whether this was due to the lack of suitable deposition sites, or whether it was an attempt to avoid cannabalism of eggs, is uncertain. In both habitat types eggs were deposited beneath water and there was no indication of extra-aquatic development as seen in *G. maculatus* and *G. fasciatus* (McDowall, 1968; Pollard, 1971a; Mitchell and Penlington, 1982).

Embryonic development has been described for very few galaxiids: Hopkins (1971) described the development of *G. divergens*; Benzie (1968a, b) the development of *G. maculatus* and *G. vulgaris* and Campos (1972) the development of *Brachygalaxias bullocki*. Time to hatching in galaxiids proved variable and highly dependent upon temperature. Benzie (1968b) found that *G. maculatus* took only 10 days to hatch at 17° C, while *B. bullocki* took 14-16 days to hatch at between 9 and 14° C (Campos, 1972). A single *G. divergens* egg hatched in 25 days at a mean temperature of 13.8° C (Hopkins, 1971). *G. truttaceus* took about 28 days to hatch in the laboratory at 12° C, while it was estimated that eggs in the field took between 4 and 6 weeks to hatch at lower temperatures.

In comparison to some fish, galaxiids hatch at a relatively advanced stage of development, with well developed mouths and pectoral fins, darkly pigmented eyes and small yolk sacs. Feeding begins after several days, and by this time the yolk has been depleted. The size of newly hatched galaxiid larvae range from about 5 mm in *G. pusilla* (Humphries, 1986) to more than 9 mm in *G. fasciatus* (Hopkins, 1979a). *G. truttaceus* larvae, being between 6.5 and 9.5 mm, are therefore quite large.

All four populations of *G. truttaceus* exhibited slow growth in length over winter, and faster growth over summer, a trend also seen in other galaxiids (Pollard, 1971a). Greater growth in length, however, occurred at FLC over winter than at CL or IL. This was probably due to lower water temperatures over winter in the lakes which would reduce metabolic processes, including assimilation of food, and the fact that lake fish, especially females, must continue to provide energy for expanding gonad over winter (see Chapter 4).

Although growth patterns of females and males could not be determined separately, growth for pooled sexes indicated important differences between populations. A faster growth rate was shown at IL than for FLC and CL and was reflected in larger sizes of age 2, although age 3 fish from both

lakes were larger than FLC fish at the same age. Increased productivity in the lake habitats may account for these differences. High productivity is generally correlated with faster growth rates in fish (Elliott, 1972a, b), and has been found to be associated with high conductivity water (Mills, *et al.*, 1983; Mann, *et al.*, 1984). The growth of *G. truttaceus* from AC, CL and IL could not be described by the Von Bertalanffy growth formula, due to relatively constant growth with age. Uniform increase in length with age is not uncommon in fisheries biology. Ricker (1975) described a similar occurrence for walleye (*Stizostedion vitreum*) and Shafi and Maitland (1971) demonstrated that both male and female perch (*Perca fluviatilis*) from two lochs in Scotland had constant growth with age. Shafi and Maitland (1971) had difficulty in accounting for the pattern of growth in perch, but emphasized the influence of temperature on growth.

In its first year of life *G. truttaceus* grew from about 8.5 mm to about 64 mm, an increase of greater than 55 mm. *G. divergens,* being a smaller species, was found to grow only 30 mm in its first year (Hopkins, 1971) while *G. maculatus* in Lake Modewarre grew about 80 mm in its first year (Pollard, 1971a). Most studies of galaxiid growth have proved difficult due to the lack of discrete size groups representing age groups. Generally, young-of-the-year galaxiids have been followed through two years of life, at which time they tended to merge with older fish (Hopkins, 1971; Eldon, 1979b; Pollard, 1971a).

An example of how differences within otherwise similar habitats may affect life history characters is the nature and extent of infestations with parasites. FLC and IL fish had relatively heavy infestations with metacercarial trematodes, more so than AC and CL, and for all populations the extent of infestations increased with body size and age. Although metacercaria did not appear to affect the fish host directly, the "black spot" caused by pigment surrounding the cysts in lake fish did make them more conspicuous and may make them more susceptable to predation from birds or brown trout, *Salmo trutta.* Adult cestodes in the body cavity did have a direct effect on reproduction in lake fish, even to the extent whereby some heavily infected individuals totally failed to develop gonads. Cone and Anderson (1977) found that several parasites of the pumpkinseed, *Lepomis gibbosus* in a lake in Ontario, Canada, increased in number with age and a number of studies have documented the effect of parasites on the development of gonad. Pearse and Timm (1971) suggested that nemotodes in the sea urchin *Centrostephanus coronatus* suppressed gametogenesis by blocking the passage of hormones, and Paperna (1974) found that infections by nematodes of fish in Lake Victoria could totally destroy gonad and prevent reproduction.

CHAPTER 4 PROXIMATE ANALYSIS

4.1 INTRODUCTION

Fisher (1958) argued that a knowledge of how much energy is devoted to reproduction in relation to the amount of energy devoted to maintenance and other non-reproductive activities should provide an insight into the evolution of life histories. By investigating the seasonal fluctuations in proximate constituents of the soma and gonad, especially fat, it is possible not only to determine which constituents are involved in reproduction, but it is also possible to make an estimate of reproductive investment in energetic terms.

Stored fat is the major energy reserve for many fish species (Love, 1970; Shul'man, 1974) and the depletion of this store during gonadal development has been the subject of several studies. Some fish, such as *Gadus morhua* store fat in the liver (Jangaard *et al.*, 1967), whilst others store their major reserves in somatic tissues (Idler and Bitners, 1959; Newsome and Leduc, 1975).

It is well documented that female fish generally produce a relatively greater quantity of gonad than males and that ovaries and testes may possess different proportions of water, fat, protein and ash (Love, 1970). It has also been shown that many species of fish transport fat, protein and other constituents from their somatic tissues and liver to supply an expanding gonad during maturation. Therefore, it is logical to presume that, due to different requirements for gonadal development, differential depletion of body reserves may occur between sexes. The greater energy requirements of the ovaries may deplete body reserves to such an extent as to be detrimental to the survival of females (Newsome and Leduc, 1975). However, spawning activities, such as nest building and agonistic interactions, may also necessitate the metabolism of large amounts of fat from the soma by males.

The relationships between individual body constituents, and between constituents and body size and condition, have been extensively investigated (see Love, 1970 for review) and various patterns have emerged. This has enabled the estimation of one constituent from the knowledge of another, or from a measure of body size (Groves, 1970; Elliot, 1976; Craig, 1977). The majority of proximate analysis studies have been conducted on commercially important species, such as *G. morhua* (Dambergs, 1964; Jangaard *et. al.*, 1967), *Coregonus* spp. (Dabrowski, 1982, 1983, 1985), *Salmo trutta* (Elliot, 1976) and *S. gairdneri* (Weatherley and Gill, 1983) or on species which undergo starvation during spawning migrations, such as several lamprey species (Beamish *et.al.*, 1979; Beamish and Legrow, 1983; Bird and Potter, 1983; Heikkala, *et al.*, 1984). Apart from the study by Craig (1977) there have been relatively few studies investigating body composition of teleosts purely for natural history purposes. Commercially important fish have received the most attention, as a knowledge of the composition of the flesh of such species, and how it varies seasonally, is important in determining the fish's marketability.

Studies of the biology of the Galaxiidae remain scarce and, to date, no studies of the body composition of galaxiids have been performed. This is probably because the only commercial use for galaxiids is when they are juveniles, *i.e.* as whitebait (McDowall and Eldon, 1980).

This chapter presents data on the proximate analysis of the soma and gonad of *Galaxias*

truttaceus from FLC, CL and IL. Fluctuations in water, fat, protein and ash of somatic tissues were followed throughout the reproductive cycles of both sexes and compared with non-reproductive individuals. The relationships between individual constituents and between body composition and body size were also investigated. Gonadal composition during maturation is described and the effects of this on the somatic tissues are discussed. Energy values are derived from proximate composition and reproductive investment, in terms of the potential energy in gonad in relation to that of the total body, is examined in the light of differences in life history traits between localities.

4.2. MATERIALS AND METHODS

4.2.1 Preparation of Samples

Samples of between 15 and 30 fish were collected from FLC, CL, and IL bimonthly during spring and summer in 1985 and monthly, 4 months prior to the populations' respective breeding seasons in 1986. No post-spawning sample was taken from FLC and analysis of testes in September 1986 for lake males was limited due to the presence of individuals of uncertain reproductive status (*i.e.* whether thay were part-spent or ripe [stage V]). The September 1985 sample for CL fish was taken before fish had spawned, whilst for IL fish this sample was taken soon after fish had spawned. For a detailed account of sampling procedure see Chapter 2. Fish were preserved in 10% neutral buffered formalin for between 24 and 96 h. The standard lengths of fish were measured to the nearest 0.1 mm using vernier calipers and fish were weighed to the nearest 10 mg. The gut and gonad were removed and weighed to the nearest 10 mg and fat attached to the gut replaced in the body cavity. The gut was not included in the proximate analyses.

The whole tissue was wet weighed and then oven dried at 65° C, for at least three days, to constant weight. The water content was calculated from the difference between the wet weight and dry weight of the tissue. Studies have shown that drying at higher temperatures oxidises lipids in the tissues which cannot, therefore, be extracted (Love, 1970). Freeze drying was tried as a means for determining the water content of fish, but was quickly abandoned in favour of heat drying as it caused the tissue to become leathery which confounded the grinding of the tissue for subsequent analyses.

Only fish which possessed somatic tissue of a minimum dry weight of 300 mg and only those gonads which had a minimum dry weight of 300 mg were analysed further for fat, protein and ash. The carbohydrate content was not determined because many workers have found that it does not constitute more than 2% by dry weight of fish (Vinogradov, 1953; Black, 1958; Craig, 1977; Love, 1980), and because most of the glycogen in fish is either utilised rapidly after ingestion or converted to and stored as fat (Hainsworth, 1981).

The dry tissue was weighed and then ground to a powder using a mortar and pestle. Three accurately weighed subsamples of *c.* 100 mg were used for fat, protein and ash determinations.

4.2.2 Fat

Procedure A number of techniques are available for the analysis of crude fat. Some of the most widely used are the Soxhlet, ethyl/ether and methanol/chloroform extractions. Although the Soxhlet fat-extraction technique has been utilised widely (Medford and Mackay, 1978) the quicker and more facile chloroform/methanol extraction (Hanson and Olley, 1963) has been favoured in recent years (Elliot, 1976; Craig, 1977; Weatherley and Gill, 1983; Long, 1985). Craig (1977) compared the Soxhlet and choroform/methanol methods for determining the fat content of *Perca fluviatilis* and found no significant differences.

The technique used in the present study is a modification of the chloroform/methanol extraction described by Craig (1977). The amount of tissue used for extraction was 5% of the amount used by Craig (1977) with the solvent volumes reduced to 25%. An accurately weighed tissue sample of *c.* 100 mg was placed in the cup of a top-drive 'Sorvall Omni-mix' blender and 4 ml water, 10 ml methanol and 5 ml chloroform were added. The cup was kept in an ice-water bath throughout the procedure. The resulting mixture was homogenised for 30 sec and a further 5 ml chloroform were then added. After homogenising for a further 30 sec, 5 ml of water were added and the solution again homogenised for 30 sec. The homogenate was then placed in two centrifuge tubes and centrifuged at 5000 rpm for 20 min. After centrifugation, the layers separated into a lower chloroform/fat layer and an upper methanol/water layer, separated by a tissue pad. A 5 ml volumetric pipette was pushed through the pad and 5 ml of the chloroform/fat layer was removed. This aliquot was placed in a weighed 50 ml conical flask and warmed at *c.* 30° C (to evaporate the chloroform) to a constant weight, leaving a residue of fat. Assuming the complete homogenisation of the fat within the chloroform, the weight of fat remaining in the conical flask was used to calculate the percentage contribution of fat to the original sample.

Reproducibility of Results Samples of somatic tissue and ovary from one fish and testis from another were dried, ground to a powder and then each divided into six parts. From each part 100 mg of tissue were taken and the resulting subsamples analysed for fat using the methanol/chloroform method.

The results indicated an increasing reproducibility with increasing fat content of the tissues (Table 4.1). The variations about the means are comparable to those of similar studies (Medford and Mackay, 1978).

Efficiency of Extraction Somatic tissue samples from nine fish were extracted. The residual tissue, and the methanol/water layer from the nine samples were pooled and filtered through filter paper and the collected material dried in a desiccator. The extraction was repeated on the collected material and it was found that the first extraction had been 96.3% efficient.

Accuracy Fat was extracted from four samples of *c.* 100 mg of pure peanut oil, assuming 100% fat, using the methanol/chloroform technique. Results indicated a ($\bar{x}\pm$SE) 98.6±1.3% return and were deemed sufficiently accurate not to require a conversion factor to be used in subsequent analyses.

Table 4.1 Fat content (%) by dry weight of replicates of somatic, ovarian andtesticular tissue.

Replicate	Fat content (%)		
	Soma	Ovary	Testis
1	11.67	19.79	13.60
2	10.61	20.84	13.63
3	8.92	20.08	15.48
4	8.63	20.49	13.07
5	9.60	21.16	12.78
6	10.66	20.56	13.57
$\bar{x}$±SE	9.94±0.46	20.49±0.20	13.69±0.38
CV	11.37	2.44	6.87

4.2.3 Protein

Procedure Protein determination of animal tissues is generally performed indirectly by analysing for total nitrogen and multiplying by a coversion factor to obtain an estimate of protein concentration (Craig, 1977). However, some workers have preferred to estimate protein directly using one of a number of colorimetric and other protein determination techniques (Groves, 1970 - Dumas method using a "Coleman" Model 29 N_2 analyser; Craig, 1977 - 'Hewlett Packard' F. and M. Scientific 185 C,H,N, analyser; Medford and Mackay, 1978 - 'Heraeus' micro-rapid N_2 gas analyser; Dawson and Grimm, 1980 - Biuret and inference methods; Delahunty and de Vlaming, 1980 - Biuret method; Dabrowski, 1982 - Biuret method).

Some workers have estimated protein by subtracting the amount of fat and ash from the sample weight, and assuming the balance to be protein (Dawson and Grimm, 1980; Reznick, 1980). In the present study a 'Kjeltec' micro-Kjeldahl total nitrogen analyser was used and a conversion factor of 6.25 was subsequently applied to calculate protein concentration (Niimi, 1974).

An accurately weighed sample of *c.* 100 mg dried ground tissue was placed in a large tube and 5 ml concentrated sulphuric acid were added. After 30 sec agitation, one half of a Kjeldahl selenium catalyst tablet was added and an inverted round-bottom flask was placed in the digestion tube to act as a stopper. The tube was heated on a hot plate at 100° C for between 3 and 5 h, after which time the mixture should have been clear for at least 30 min.

The tubes were left to cool and then 50 ml distilled water were added. The tube containing the digest was placed in the 'Kjeltec' distillation unit and 25 ml of 40% sodium hydroxide solution were added. Steam was bubbled through the mixture and the distilled ammonia/water mixture was condensed in a water-cooled condenser and received in a conical flask containing 20 ml 0.1 N boric

acid, bromocroesol green/methyl red indicator solution (Association of Official Analytical Chemists, 1969). Approximately 100 ml of distillate were collected, after which the conical flask was removed from the distillation unit and the solution was titrated with 0.1 N hydrochloric acid until the endpoint was reached. The volume of acid (a) less a blank titration correction volume (b) was substituted into the following equation:

$$\%N = \frac{(a-b) \times 14.008 \times \text{Normality of acid}}{\text{weight of sample (g)} \times 10}$$

The concentration of hydrochloric acid was determined by titration against a commercially available standard 0.1 N sodium hydroxide.

Table 4.2 Protein content (%) by dry weight of replicates of somatic,ovarian and testicular tissue.

Replicate	Protein content (%)		
	Soma	Ovary	Testis
1	71.08	64.73	91.28
2	74.64	64.68	89.47
3	74.13	65.10	90.60
4	71.96	65.34	91.01
5	72.92	65.07	90.51
6	70.30	63.79	85.79
$\bar{x}$±SE	72.51±0.70	64.79±0.22	89.78±0.84
CV	2.35	0.84	2.28

Reproducibility of Results Samples of somatic, ovarian and testicular tissue, each from a separate fish, were dried, ground to a powder and then each was divided into six parts. Six accurately weighed subsamples of *c.* 100 mg were taken from each tissue sample and analysed using the Kjeldahl total N method as described above. The coefficients of variation for the replicates of soma, ovary and testis were 2.35, 0.84 and 2.28 respectively (Table 4.2).

Accuracy The accuracy of the Kjeldahl total N method was estimated by analysing commercially available bovine serum albumin (BSA) with a known N content (16.3%). Three accurately weighed samples of *c.* 100 mg were used in the analysis. The results show a consistent underestimation of the nitrogen and therefore protein content (-7.7%) of the BSA (Table 4.3), which has been found by other workers utilising the same equipment (Avanthi, pers. comm.). A correction factor of 1.084 ($^{98.99}/_{91.29}$) was subsequently applied to all protein values used in further analyses.

Table 4.3 Kjeldahl total N determination of bovine serum albumin (98.99% protein)

Sample number	Protein (%)
1	91.38
2	91.29
3	91.21
$\bar{x}$±SE	91.29±0.05

4.2.4 Ash

Procedure An accurately weighed sample of *c.* 100 mg dried ground tissue was placed in a preweighed crucible, without lid, and heated at 500° C in a muffle furnace for at least 12 h. The crucible was cooled in a desiccator and then weighed. The weight of the residue was the weight of the ash.

Validation To determine if material was lost due to combustion of the tissue sample when it was placed in a preheated oven, four samples were each divided into halves; one half from each sample was heated gradually to 500° C over several hours and the other half was ashed with the oven having previously been heated to 500° C. The results indicated no difference in ash content between the two methods (paired t-test, df_3, t=0.56, p>0.1) (Table 4.4). The precision of the analysis was tested using these data and showed small standard errors for each of the four lots of two replicates (Table 4.4). No standard was available to test the accuracy of the ashing technique.

Table 4.4 Ash values for a comparison of methods of ashing by gradually heating oven or using a preheated oven.

	Ash content (%)		
Sample number	Gradual heat increase	Preheated oven	$\bar{x}$±SE
1	8.95	8.79	8.87±0.08
2	8.62	8.69	8.66±0.03
3	8.47	8.09	8.28±0.02
4	8.63	9.95	9.29±0.66
$\bar{x}$±SE	8.67±0.10	8.88±0.27	

4.2.5. Effect of Preservation of Samples

The effect of preservation in formalin on the proximate analyses was determined. A sample of eighteen fish was collected and divided into two approximately equal groups with respect to sex and size composition (sex could not be determined prior to dissection). The fish were anaesthetised in 2% 'benzocaine' and one group preserved in 10% neutral buffered formalin for 96 h, while the other group was analysed fresh.

Both groups were dried at 65° C and analysed for proximate components. Table 4.5 shows the means and standard errors for each analysis and the results indicate that the differences between the means for unpreserved and preserved samples were within the errors associated with the respective analyses.

4.2.6 Bomb Calorimetry

It is common practice to convert the components of body tissues into energy terms (Craig,1977); however, it is essential to first validate this conversion. Bomb calorimetry was carried out on the 9 unpreserved dried, ground somatic tissue samples referred to above and proximate analyses were carried out on the 9 preserved dried ground tissue samples referred to above. The micro-bomb calorimeter was constructed from a design by Phillipson (1964). Three accurately weighed replicates of *c.* 15 mg were analysed for each tissue sample by bomb calorimetry. (Table 4.6).

The factors used here for the conversion of proximate components to energy content are based on the heats of combustion of common fatty acids and amino acids; they are 39.5388 kJ/g for fat and 23.6396 kJ/g for protein (Kleiber, 1961). There was no significant difference between the values obtained from the bomb calorimeter and those obtained by the application of these conversion factors (Table 4.6; paired t-test, df_8, $t=1.11$, $p>0.1$). This agrees with other studies (*e.g.* Craig, 1977; Dawson and Grimm, 1980) although discrepancies have sometimes been found between energy values derived from bomb calorimetry and proximate analusis. It has been suggested that different fatty acids may be present with different heats of combustion, or that the lipid content is being overestimated to some degree (Craig, 1977).

4.2.7 Statistical Analysis

Somatic Composition In the analysis of the body composition of somatic and gonadal tissues of *G. truttaceus* from the three populations, fish were divided into two groups. The first group consisted of fish of both sexes which were classified as being at maturity stages I and II (see Section 3.2.3) and are referred to as 'non-reproductives'. This group consisted of juveniles, non-reproductive older fish and adults prior to the commencement of maturation. The second group consisted of all fish classified as being at maturity stages III through VI and will be referred to as 'reproductives'. This second group was further separated by sex, while for the non-reproductive

Table 4.5 Composition (% fat, % protein, % ash) by dry weight of somatic tissues of preserved and unpreserved fish.

	Proximate constituent							
	Fat (%)		Protein (%)		Ash (%)		Total (%)	
	Unpres.	Pres.	Unpres.	Pres.	Unpres.	Pres.	Unpres.	Pres.
	4.43	4.06	79.13	79.57	13.75	13.34	97.41	96.97
	5.95	3.37	84.89	77.25	12.14	11.91	102.98	92.53
	6.72	6.08	84.34	79.84	9.16	8.22	100.22	94.14
	4.20	6.53	82.19	81.53	10.41	7.01	96.90	95.07
	2.96	3.17	81.36	79.49	13.89	9.09	98.21	91.75
	5.55	13.78	83.16	79.35	9.82	7.29	98.53	100.42
	14.64	18.36	78.57	76.20	8.43	7.56	101.64	102.12
	8.93	15.75	80.85	77.23	9.98	7.14	99.76	100.12
	15.46	19.03	77.65	74.48	8.72	6.51	101.83	100.02
$\bar{x}$	7.65	10.01	81.35	78.33	10.71	8.67	99.72	97.01
SE	1.49	2.08	0.84	0.73	0.70	0.80	0.71	1.27
Difference between means	+2.36		-3.02		-2.04		-2.71	

Table 4.6 Mean energy values ±SD for somatic tissues obtained by direct calorimetry and conversion of proximate constituents, using conversion factors of 39.5388 kJ / g for fat and 23.6396 kJ / g for protein. (Three subsamples from each tissue sample were used in direct calorimetry and a single subsample from a different fish used in each proximate analysis)

	Method of analysis	
	Calorimetry (kJ / ash free g)	Proximate Analysis (kJ / ash free g)
	21.256±0.583	18.894
	18.667±1.348	20.815
	19.759±2.013	20.999
	18.974±0.377	19.574
	20.881±0.584	18.865
	19.988±0.677	20.280
	19.942±0.438	22.875
	20.659±1.930	21.114
	19.728±0.309	22.999
Total $\bar{x}$±SE	19.974±0.280	20.713±0.500

group, the sexes were pooled. To justify the pooling of male and female non-reproductives, t-tests were performed within each site between sexes for all body constituents expressed as percentages. In only two cases were there significant differences between sexes (FLC, August 1985 for protein, $0.01<p<0.05$; FLC, December 1985 for protein, $0.01<p<0.05$). Sample sizes for proximate analysis of somatic tissues are shown in Table 4.7.

Absolute weights of body constituents were checked for normality and logarithmically transformed if necessary (Zar, 1974). Regression analyses were performed between absolute weight of constituent and body weight and the value for a standard fish weighing 20 g (see Section 2.1.7) substituted into the equations for comparative purposes (see Craig, 1977 and Medford and MacKay, 1978 for a similar method of analysis). Where small sample sizes existed for a particular month and/or the constituent weight / body weight relationship was not significant, the mean of all values was taken and used in analyses. Regression equations were compared by the use of analysis of covariance (ANCOVA) following the procedure described in Section 2.1.7; means and regressions were never compared statistically. Standard errors for somatic constituents are derived as per Section 2.1.7

Somatic constituents expressed as percentages of dry weight were checked for homogeneity of variance and arcsin transformed and regressed against standard length to determine the effects of body size and age. In the analysis of relationships between individual constituents, values were expressed as percentages of dry weight of tissues and arcsin transformed (Zar, 1974).

Gonadal Composition The gonad weight of a standard fish (20 g) within each monthly sample and from each locality was derived from regressions of gonad weight on body weight (see Section 3.3.3). The absolute weight of gonad constituents was calculated by multiplying the gonad weight of the standard fish by the percentage composition for that particular constituent. Sample sizes for gonadal composition are shown in Table 4.8. Gonads were not sufficiently large to analyse for fat, protein and ash until February 1986, although the gonadal water content was determined from October 1985. No analyses of gonads for stage VI (spent) fish were possible for the same reason.

Reproductive investment was calculated from regressions of total absolute energy of the gonadal tissues against absolute energy in the body (soma + gonad) and the value for a standard fish substituted into the equations. The total body energy value for a standard fish was taken as the mean of all absolute total body energies for reproductive fish from FLC, CL and IL (110 kJ).

4.3 RESULTS

4.3.1 Somatic Fat

Peaks in absolute somatic fat in reproductive females from all localities occurred in late summer/early autumn (Fig. 4.1.a). Lake females attained peaks in excess of that of FLC females (ANCOVA - FLC/CL: for slopes $df_{10,1}$, $F=29.69$, $p<0.001$; FLC/IL: for slopes $df_{10,1}$, $F=5.53$, $p<0.05$) and CL females attained a higher level than IL females (ANCOVA - for slopes $df_{10,1}$, $F=45.89$, $p<0.001$) after females from all sites had possessed lower levels over winter. Depletion of

Table 4.7 Sample sizes for proximate analysis of somatic tissues. (F = reproductive females - Stage III to VI; M = reproductive males - Stage III to VI; NR = non-reproductives - Stage I and II; a dash indicates none in sample; * denotes those samples for which only water was analysed due to small size of fish)

		Category		
Locality	Month	F	M	NR
FLC	vii.85	7	–	7
	viii.85	7	2	6
	x.85	6	7	11
	xii.85	5	–	14
	ii.86	7	10	2
	iii.86	15	9	5
	iv.86	11	9	7
	v.86	14	4	5
CL	vii.85	–	–	–
	viii.85	–	–	–
	ix.85	7	2	5
	x.85	7	6	2
	xii.85	7	–	13
	ii.86	7	6	1
	iii.86	9	4	1*
	v.86	6	3	1
	vi.86	6	6	3*
	viii.86	9	5	3
	ix.86	1	–	–
	x.86	1	3	4
IL	vii.85	8	5	2
	viii.85	–	–	–
	ix.85	7	5	4
	x.85	6	5	4
	xii.85	10	–	10
	ii.86	9	9	2*
	iii.86	7	7	1*
	v.86	6	6	3
	vi.86	9	5	1*
	viii.86	19	9	3
	ix.86	12	7	7
	x.86	13	3	2

Table 4.8 Sample sizes for proximate analysis of gonadal tissues.
(F = female; M = male; a dash indicates gonads too small to analyse)

Locality	Date	Analysis							
		Fat		Protein		Ash		Water	
		F	M	F	M	F	M	F	M
FLC	x.85	–	–	–	–	–	–	6	7
	xii.85	–	–	–	–	–	–	5	–
	ii.86	4	–	4	–	4	–	7	9
	iii.86	9	5	9	5	9	5	15	9
	iv.86	11	5	11	5	11	5	11	9
	v.86	3	–	3	–	3	–	14	3
CL	x.85	–	–	–	–	–	–	6	6
	xii.85	–	–	–	–	–	–	7	–
	ii.86	5	5	4	5	5	5	7	6
	iii.86	8	4	7	4	8	4	9	4
	v.86	6	–	6	–	6	–	6	3
	vi.86	5	5	6	5	6	5	6	6
	viii.86	8	4	9	4	9	4	9	5
	ix.86	1	–	–	–	–	–	1	1
IL	x.85	–	–	–	–	–	–	6	5
	xii.85	–	–	–	–	–	–	10	–
	ii.86	5	1	5	1	5	1	9	9
	iii.86	6	7	6	7	6	7	7	7
	v.86	6	5	6	4	6	4	6	6
	vi.86	9	5	9	5	9	5	9	5
	viii.86	19	7	19	7	19	7	19	9
	ix.86	4	–	5	–	5	–	12	7

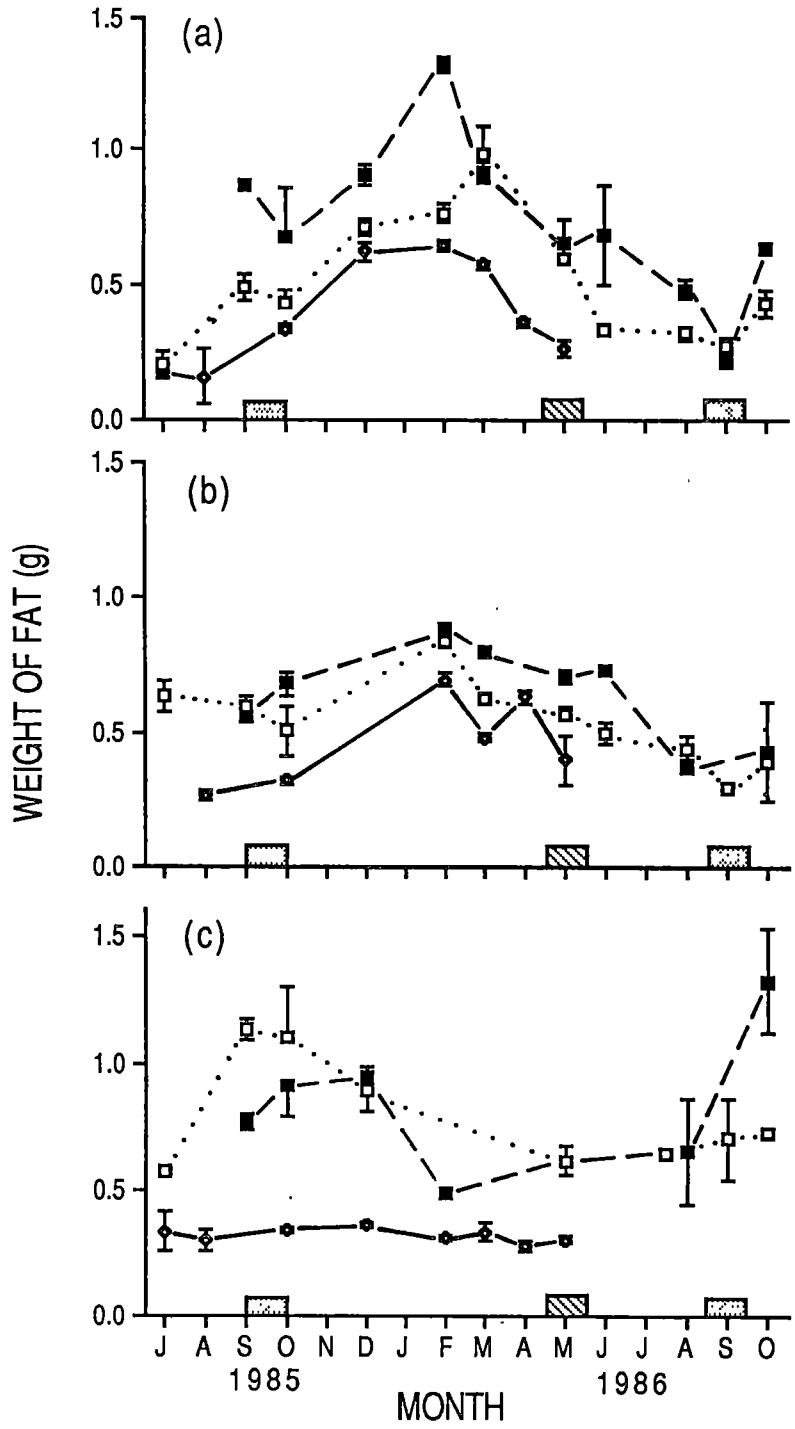


Fig. 4.1 Absolute weight ± SE of somatic fat in a standard (a) female, (b) male and (c) non-reproductive fish from FLC (diamonds), CL (closed squares) and IL (open squares). Stippled boxes indicate spawning time of lake fish; hatched boxes indicate spawning time of creek fish.

somatic fat took place at all localities during the months following the peaks. By May, when creek fish were spawning, a standard FLC female had lost 0.38 g fat, compared with 0.67 g in a CL female and 0.39 g in a IL female. Lake females continued to lose somatic fat after this time until just prior to spawning in August. By spawning time, a standard CL female had lost 1.11 g of its fat, while a standard IL female had lost 0.72 g of its fat.

Fluctuations in somatic fat levels for FLC males showed a similar pattern to those of FLC females and peak levels occurred at similar times in both sexes at all localities during the year (Fig. 4.1.b). The peaks of both sexes were of a comparable magnitude at FLC and IL, however, CL females possessed significantly more fat at their peak than CL males (ANCOVA - for slopes $df_{9,1}$, $F=5.26$, $p<0.05$). At spawning in May, a standard FLC male had lost 0.30 g fat, while at spawning in August, a standard CL male had lost 0.52 g and a IL male had lost 0.55 g fat. From these results it is apparent that from all localities the somatic tissues of females were depleted to a greater extent than those of males.

No clear pattern in somatic fat levels of non-reproductive fish was revealed (Fig. 4.1.c). FLC non-reproductives fluctuated little throughout the year, while IL and CL non-reproductives showed peaks in early spring and early summer, respectively. From highest levels of somatic fat to lowest levels, a standard FLC non-reproductive lost 0.08 g, a CL non-reproductive lost 0.45 g and an IL non-reproductive lost 0.51 g. Levels were consistently lower in FLC non-reproductives than in FLC reproductives, and at lake localities troughs in fat levels were not as pronounced in non-reproductive as in reproductive fish. In general, both reproductives and non-reproductives from FLC possessed lower amounts of somatic fat than did fish from either lake.

4.3.2 Somatic Protein

Absolute somatic protein levels of reproductive females at CL increased in late autumn/early summer (ANCOVA - October/December: ns for slopes, for elevations $df_{11,1}$, $F=22.74$, $p<0.01$) whilst levels for FLC and IL fish fluctuated with no apparently consistent pattern (Fig. 4.2.a, b). Levels increased just prior to or immediately after spawning at all localities and for reproductives of both sexes. Non-reproductives maintained relatively stable levels of somatic protein (Fig. 4.2.c), although the somatic protein of IL non-reproductives appeared to increase in October 1986 (this could not be tested statistically due to the small sample size for October). Protein levels were consistently lower for males than females from all localities.

4.3.3 Somatic Ash

Fluctuations in absolute somatic ash levels of reproductive fish from the three localities followed similar patterns (Fig. 4.3.a, b). In CL females there was an increase in weight of ash in late autumn/early summer (ANCOVA - October/December: ns for slopes, for elevations $df_{11,1}$, $F=13.35$, $p<0.01$) and an increase in ash levels for IL females in the months leading up to spawning (ANCOVA - June/August: ns for slopes, for elevations $df_{25,1}$, $F=4.50$, $p<0.05$). There were no consistent patterns in the fluctuations in somatic ash levels of reproductive males from any locality.

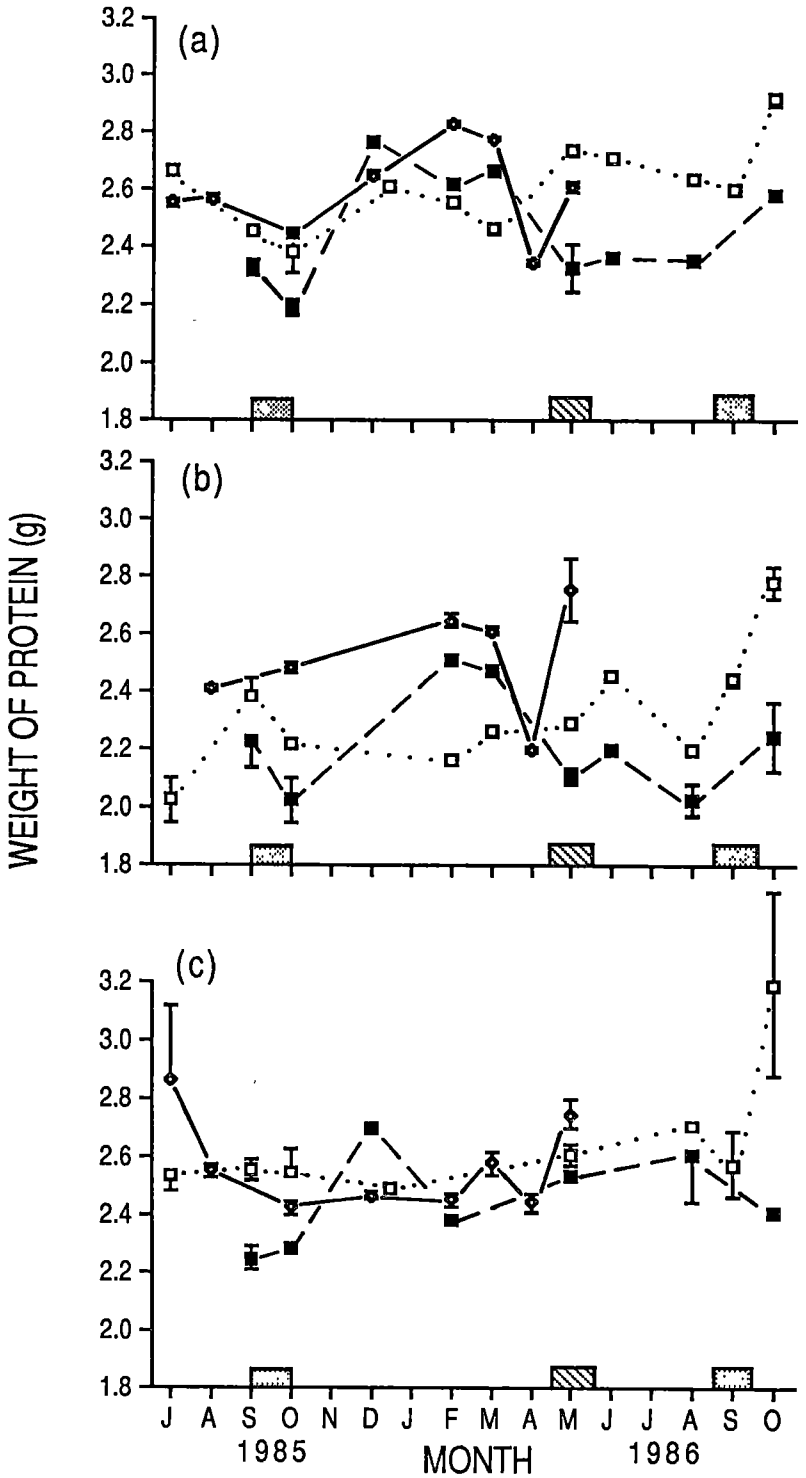


Fig. 4.2 Absolute weight ± SE of somatic protein in a standard (a) female, (b) male and (c) non-reproductive fish from FLC (diamonds), CL (closed squares) and IL (open squares). Stippled boxes indicate spawning time of lake fish; hatched boxes indicate spawning time of creek fish.

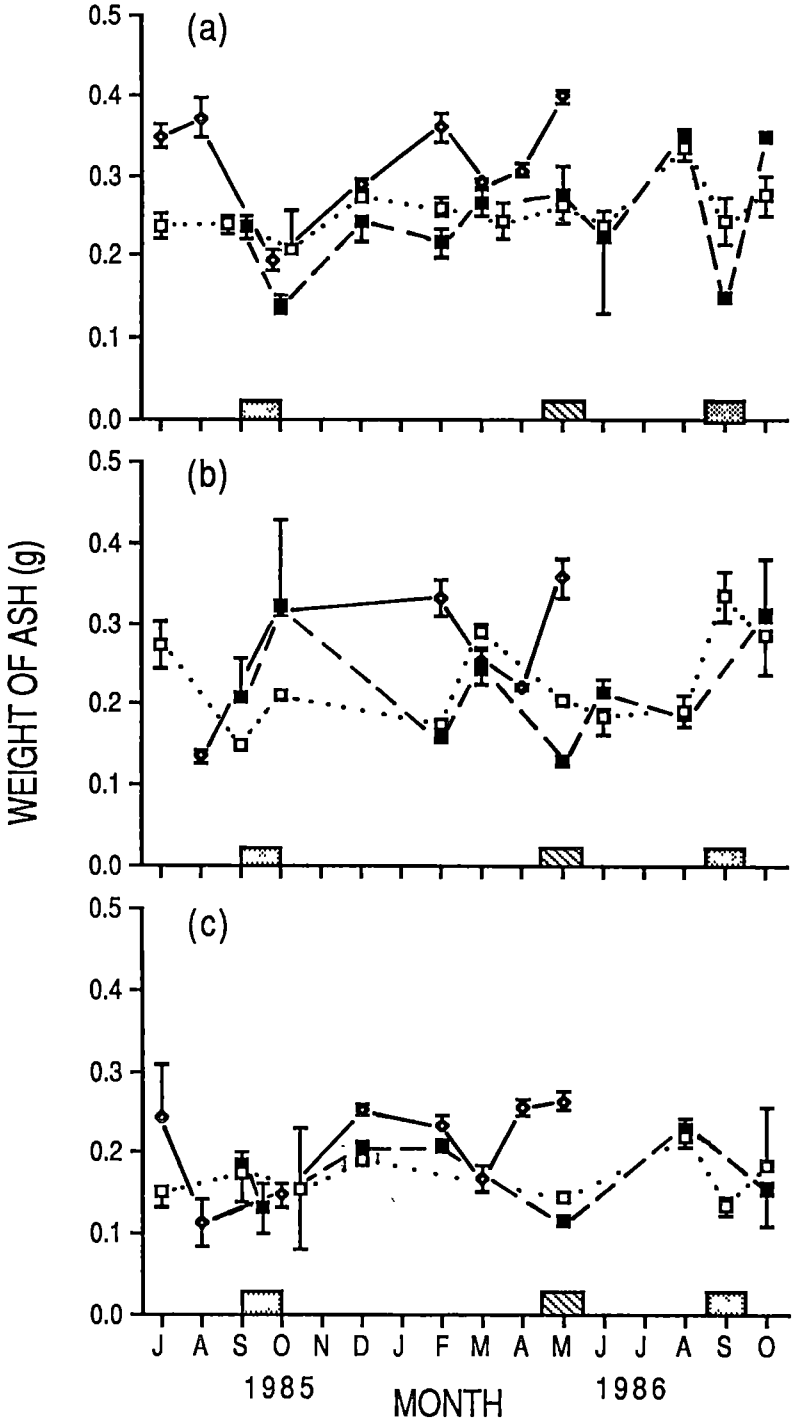


Fig. 4.3 Absolute weight ± SE of somatic ash in a standard (a) female, (b) male and (c) non-reproductive fish from FLC (diamonds), CL (closed squares) and IL (open squares). Stippled boxes indicate spawning time of lake fish; hatched boxes indicate spawning time of creek fish.

Non-reproductives showed small fluctuations throughout the year and in general had lower ash levels than did reproductives (Fig. 4.3.c). Spawning IL reproductives of both sexes had higher somatic ash levels than did non-reproductives at the same time (ANCOVA - pooled sex: ns for slopes, for elevations $df_{29,1}$, F=4.15, p<0.05), although the small sample size for non-reproductives at this time was small.

4.3.4 Somatic Water

In general absolute somatic water levels fluctuated inversely with somatic fat (Fig. 4.4), the peaks in water levels occurring at times of low fat and *vice versa*. Weights of water were lowest in summer in reproductive females from all localities (Fig. 4.4.a), whilst those of FLC and CL males were at their lowest levels in the month prior to spawning (Fig. 4.4.b). The somatic water of a standard IL male had its lowest level in winter in 1985, and did not fluctuate significantly once it had reached its peak the following spring. Non-reproductives from FLC showed no consistent trends in water levels (Fig. 4.4.c), however, water levels of CL and IL non-reproductives appeared to increase over summer, although this could not be tested statistically.

4.3.5 Gonadal Fat

The percentage composition by dry weight of the ovaries and testes are shown in Figure 4.5.a In most months where it could be tested ovaries contained a greater percentage of fat (range: 19.86% to 37.69%) than did testes (range: 9.61% to 22.54%). See Appendix 3.a for results of significance tests.

Deposition of fat in absolute terms in the ovaries initially occurred at a greater rate in FLC females than in CL and IL females (unpaired t-test - March FLC/CL: df_{15}, t=11.72, p<0.001; FLC/IL: df_{13}, t=8.52, p<0.001) (Fig. 4.6.a). However, by May FLC and IL females possessed equal amounts of gonadal fat, whilst CL females possessed a greater amount (unpaired t-test - FLC/CL: df_7, t=9.11, p<0.001; CL/IL: df_{10}, t=9.13, p<0.001). Isabella Lagoon females continued depositing fat into their ovaries into winter and by June levels were similar to those of CL females. Weights of fat in the ovaries decreased for CL females after May (unpaired t-test - May/August: df_{12}, t=5.19, p<0.001) and for females which had not spawned in September at IL, similar levels of fat were found in ovaries compared with fish collected a month previously. No differences were found in the amount of fat in the ovaries of females from all localities prior to spawning, however, at peak levels of gonadal fat lake females possessed significantly more fat in their ovaries than did FLC females (FLC/CL: df_7, t=9.11, p<0.001; FLC/IL: df_{10}, t=4.42, P<0.01).

Increases in absolute weights of fat in testes were marginal at all localities (Fig. 4.6.b). The fat in the testes of FLC males peaked at 0.09 g while CL and IL males at their peaks had 0.12 g and 0.13 g of fat in their testes, respectively. At spawning there were no differences in weights of fat in the testes of males from all localities. In absolute terms females possessed a far greater amount of fat in their gonad prior to spawning than did males (unpaired t-test between males and females - FLC: df_{14},

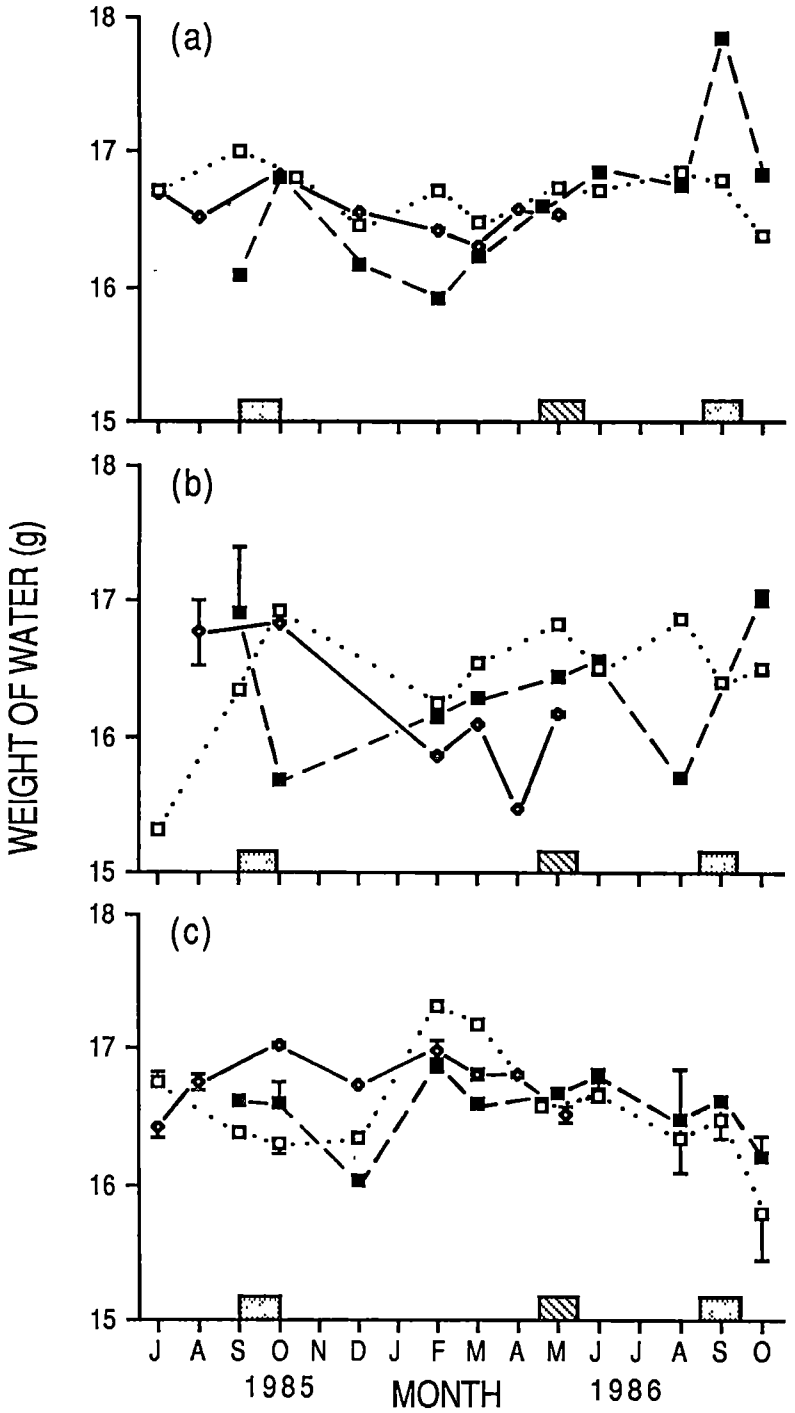


Fig. 4.4 Absolute weight ± SE of somatic water in a standard (a) female, (b) male and (c) non-reproductive fish from FLC (diamonds), CL (closed squares) and IL (open squares). Stippled boxes indicate spawning time of lake fish; hatched boxes indicate spawning time of creek fish.

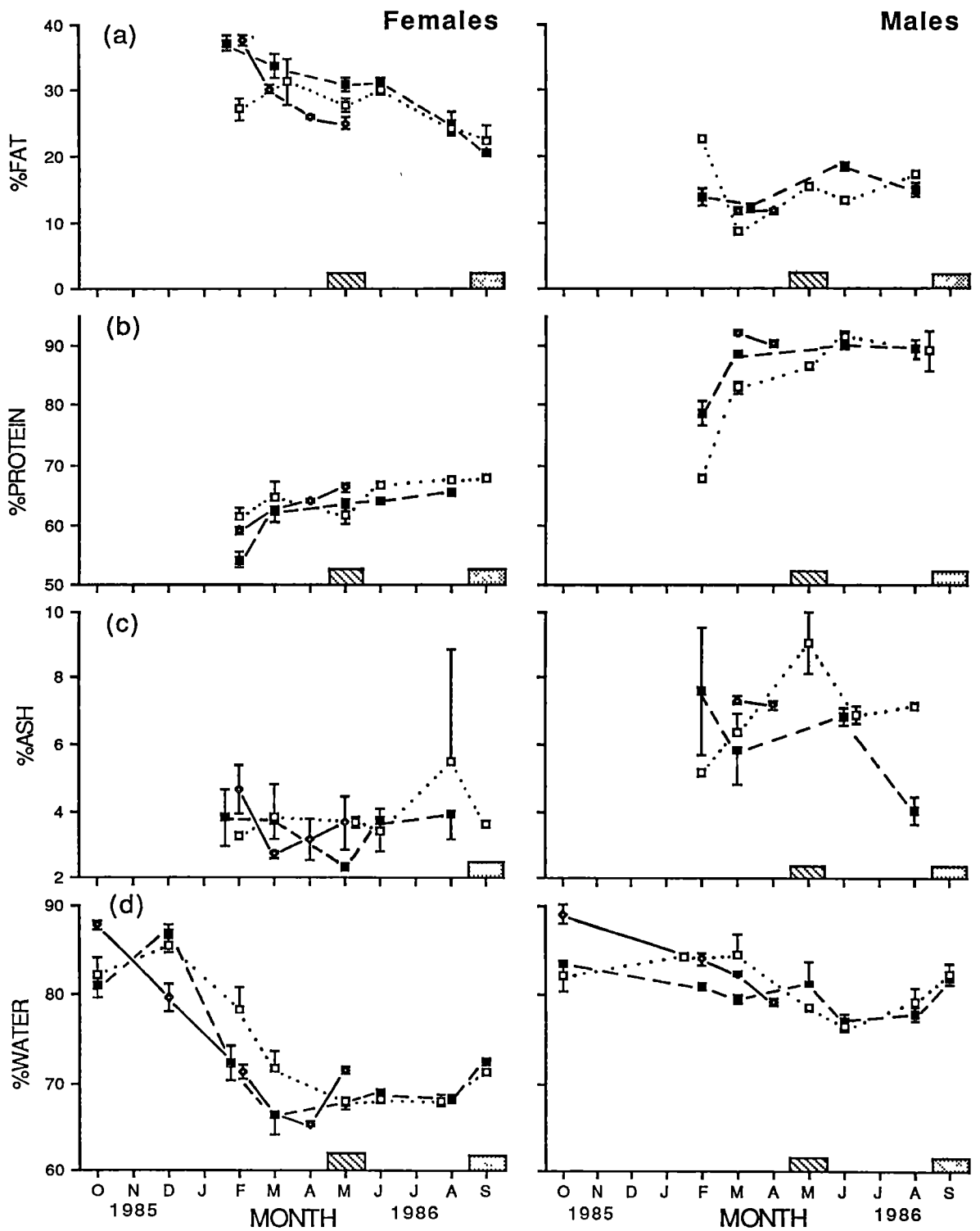


Fig. 4.5 Percentage gonadal (a) fat, (b) protein, (c) ash (by dry weight) and (d) water for females and males from FLC (diamonds), CL (closed squares) and IL (open squares). Stippled boxes indicate spawning time of lake fish; hatched boxes indicate spawning time of creek fish.

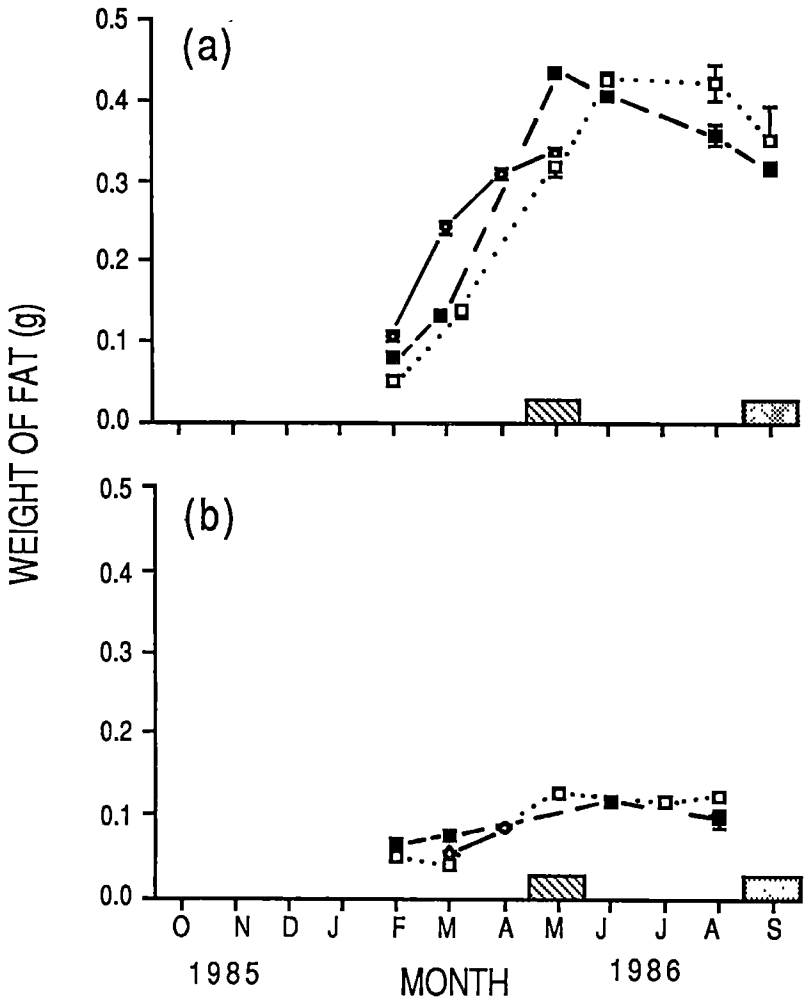


Fig. 4.6 Absolute weight of gonadal fat ±SE in a standard (a) female and (b) male fish from FLC (diamonds), CL (closed squares) and IL (open squares).Stippled boxes indicate spawning time of lake fish; hatched boxes indicate spawning time of creek fish.

t=24.34, p<0.001; CL: df_{10}, t=13.82, p<0.001; IL: df_{24}, t=7.75, p<0.001), this being partly attributable to the greater percentage of fat in ovaries and partly to the overall larger gonads of females.

4.3.6 Gonadal Protein

The percentage composition by dry weight of protein was almost always greater in testes than in ovaries (Fig 4.5.b). The percentage protein of testes ranged from 67.86 to 97.59, whilst that of ovaries ranged from 54.23 to 70.13. See Appendix 3.b for results of significance tests.

Protein was incorporated into the ovaries of FLC females initially at a greater rate than into the ovaries of lake females (unpaired t-test - March FLC/CL: df_{14}, t=9.11, p<0.001; FLC/IL: df_{13}, t=4.418, p<0.01) (Fig. 4.7.a). By May, FLC and CL females possessed similar amounts of protein in their ovaries, whilst IL females had a lesser amount (FLC/IL: df_7, t=19.97, p<0.001; CL/IL: df_{10}, t=13.44, p<0.001). Isabella Lagoon females continued to deposit protein into their ovaries after this time and, prior to spawning, possessed significantly more protein in their ovaries than did CL and FLC females (unpaired t-test - FLC/IL: df_{20}, t=5.55, p<0.001; CL/IL: df_{26}, t=5.55, p<0.001).

The absolute weights of protein in the testes of males prior to spawning were significantly less than the weights of protein in the ovaries of females at the same time (unpaired t-test - April FLC: df_{14}, t=5.85, p<0.001; August CL: df_{11}, t=13.80, p<0.001; August IL: df_{24}, t=13.68, p<0.001) (Fig. 4.7.a,b). The amount of protein in the testes of CL males did not increase significantly after March, whilst that of IL males reached a higher level than Cl males in June (unpaired t-test - df_8, t=10.80, p<0.001), but decreased afterwards, so that spawning males from CL and IL showed no differences in the amount of protein in their testes and had similar levels to FLC males.

4.3.7 Gonadal Ash

Generally the mean percentage ash by dry weight of testes for individual months was greater than it was for ovaries (Fig 4.5.c). The percentage ash in ovaries ranged from 2.74% to 5.47%, whilst that of testes ranged from 4.02% to 10.36%. See Appendix 3.c for results of significance tests.

Increases in absolute weights of ash in the ovaries of females from all localities followed a similar pattern, until FLC fish spawned (Fig. 4.8.a). After this time the amount of ash continued to increase in the ovaries of lake females, IL females showing a large increase in the month prior to spawning. Similar ash levels of the ovaries of spawning females were found from all localities.

The absolute weight of ash in the testes of CL males did not fluctuate significantly during gonadal maturation (Fig. 4.8.b). Weight of ash increased during maturation in the testes of FLC and IL males and prior to spawning there were no differences between the males from these two localities. FLC and IL males possessed significantly more ash in their testes than did CL males prior to their respective spawning times (unpaired t-test - FLC/CL: df_7, t=9.41, p<0.001; IL/CL: df_9, t=12.61, p<0.001). There were no intersexual differences in gonadal ash content at all localities prior to spawning.

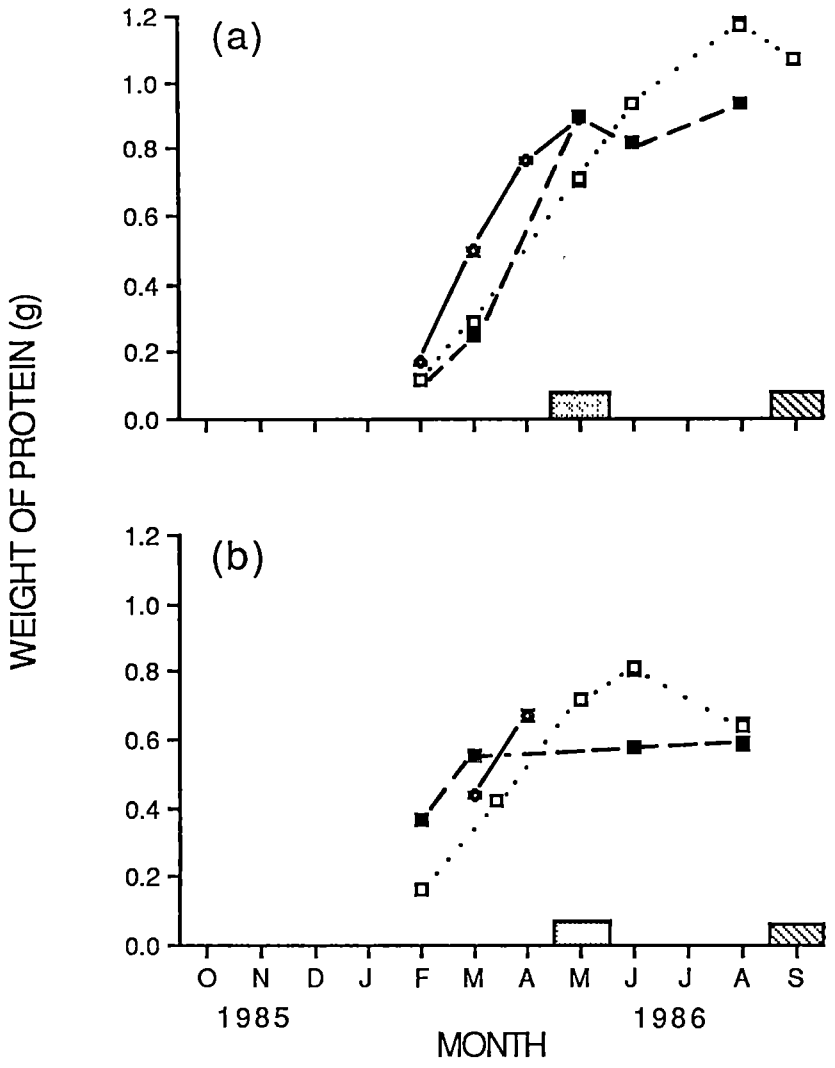


Fig. 4.7 Absolute weight of gonadal protein ±SE of a standard (a) female and (b) male fish from FLC (diamonds), CL (closed squares) and IL (open squares). Stippled boxes indicate spawning time of lake fish; hatched boxes indicate spawning time of creek fish.

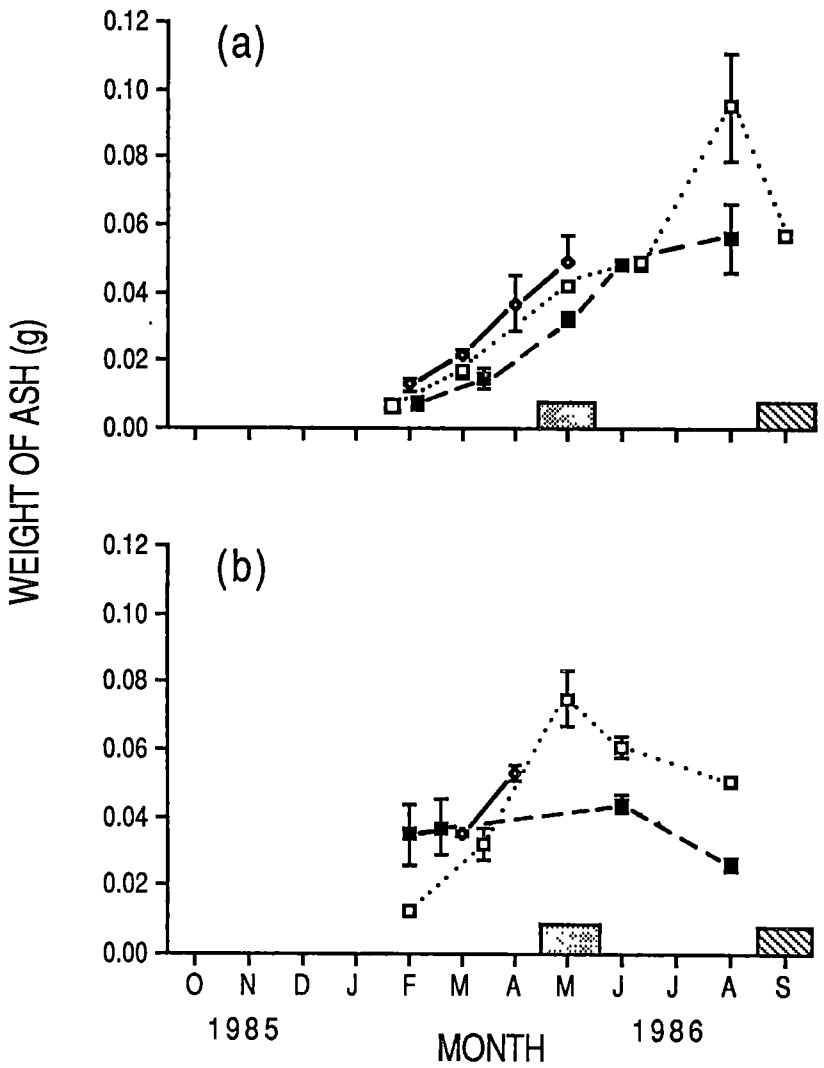


Fig. 4.8 Absolute weight of gonadal ash ±SE of a standard (a) female and (b) male fish from FLC (diamonds), CL (closed squares) and IL (open squares). Stippled boxes indicate spawning time of lake fish; hatched boxes indicate spawning time of creek fish.

4.3.7 Gonadal water

Testes generally possessed a greater percentage of water than did ovaries (Fig 4.5.d). Spawning females had ovaries with between 71.4% and 72.4% water, whilst males at spawning had testes with between 82.1% and 83.9% water. See Appendix 3.d for results of significance tests.

The amount of ovarian water began increasing after December and in February was the same in fish from all localities (Fig. 4.9.a). By May ovaries of FLC females had a greater amount of water than CL females (unpaired t-test - df_{18}, t=2.87, $p<0.05$) and IL females possessed less than both FLC and CL females (unpaired t-test - FLC/IL: df_{18}, t=6.94, $p<0.001$; CL/IL: df_{10}, t=17.03, $p<0.001$). Water continued to accumulate in ovaries of IL females after FLC fish had spawned, however, CL females did not show any increase until after August. At spawning the ovaries of IL females had significantly more water in them than those of FLC females (unpaired t-test - df_{24}, t=5.54, $p<0.001$) but, due to the small sample size for CL for this month, no further comparisons could be made.

In the early months of maturation lake males possessed a greater absolute weight of water in their gonads than did lake females (unpaired t-test - March CL: df_{11}, t=76.08, $p<0.001$; IL: df_{12}, t=24.03, $p<0.001$) (Fig. 4.9.b), whilst FLC fish of both sexes had similar amounts. The amount of water in the testes of lake males did not increase significantly after March, and at spawning males from all localities possessed similar weights of water. Females from CL and IL had greater amounts of water in their gonads than did males in the month prior to spawning (unpaired t-test - CL: df_{12}, t=22.15, $p<0.001$; IL: df_{26}, t=16.03, $p<0.001$), whilst FLC males had a greater amount of water in their gonads than females in the month prior to spawning (unpaired t-test - df_{18}, t=33.08, $p<0.001$).

4.3.8 Energy Content of Somatic Tissues

Due to the high energy content of fat, fluctuations in the energy content of somatic tissues essentially mirrored fluctuations in fat levels. (Fig. 4.10). There was an increase in energy to a peak in late summer/early autumn at all localities and for both sexes, and then a decline to spawning. The energy content of somatic tissues of a standard FLC female decreased by 4.03 kJ/g from the peak to when fish spawned (Fig. 4.10.a). A standard CL female lost 5.12 kJ/g and a standard IL female lost 4.28 kJ/g from its somatic tissues by the time fish had spawned. The equivalent loss in a standard FLC male was 3.00 kJ/g and that of a standard IL male was 3.15 kJ/g (Fig. 4.10.b), whilst in a standard CL male there was a decrease of 4.46 kJ/g. Lake females generally possessed greater somatic energy content than FLC females, although no differences in energy content of soma of fish among localities existed at their respective spawning times.

4.3.9 Energy Content of Gonadal Tissues

The energy content of ovaries decreased during the final months of maturation in fish from all localities (Fig. 4.11.a) and probably reflected the proportionally greater amount of water being shunted into the ovaries at this time. The relative energy content of ovaries at spawning was similar at all localities. In contrast to ovaries of lake females, the relative energy content of testes of lake males

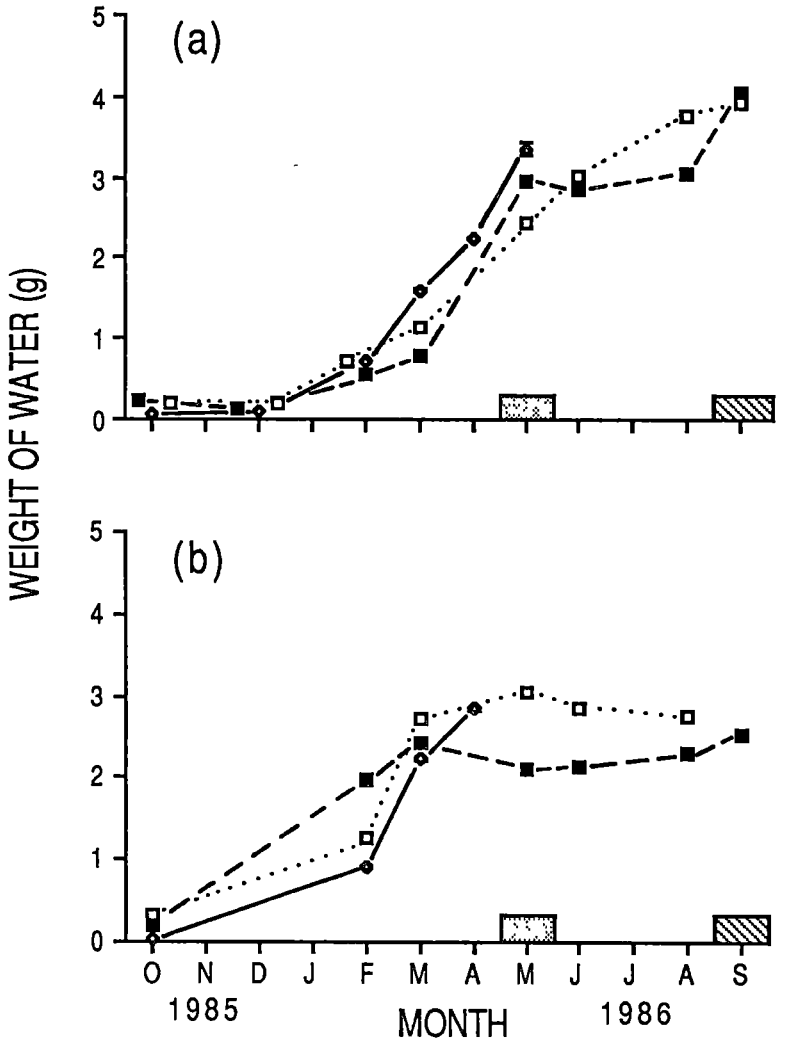


Fig. 4.9 Absolute weight of gonadal water ±SE of a standard (a) female and (b) male fish from FLC (diamonds), CL (closed squares) and IL (open squares). Stippled boxes indicate spawning time of lake fish; hatched boxes indicate spawning time of creek fish.

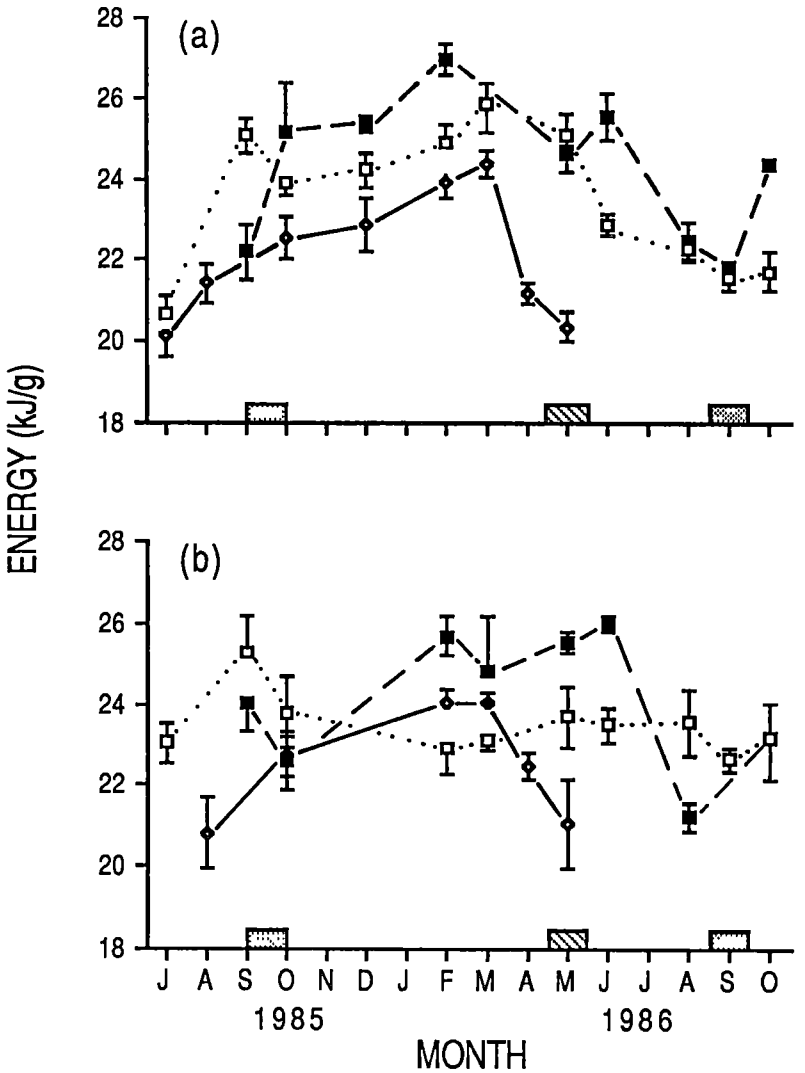


Fig. 4.10 Somatic energy ±SE of a standard (a) female and (b) male fish from FLC (diamonds), CL (closed squares) and IL (open squares). Stippled boxes indicate spawning time of lake fish; hatched boxes indicate spawning time of creek fish.

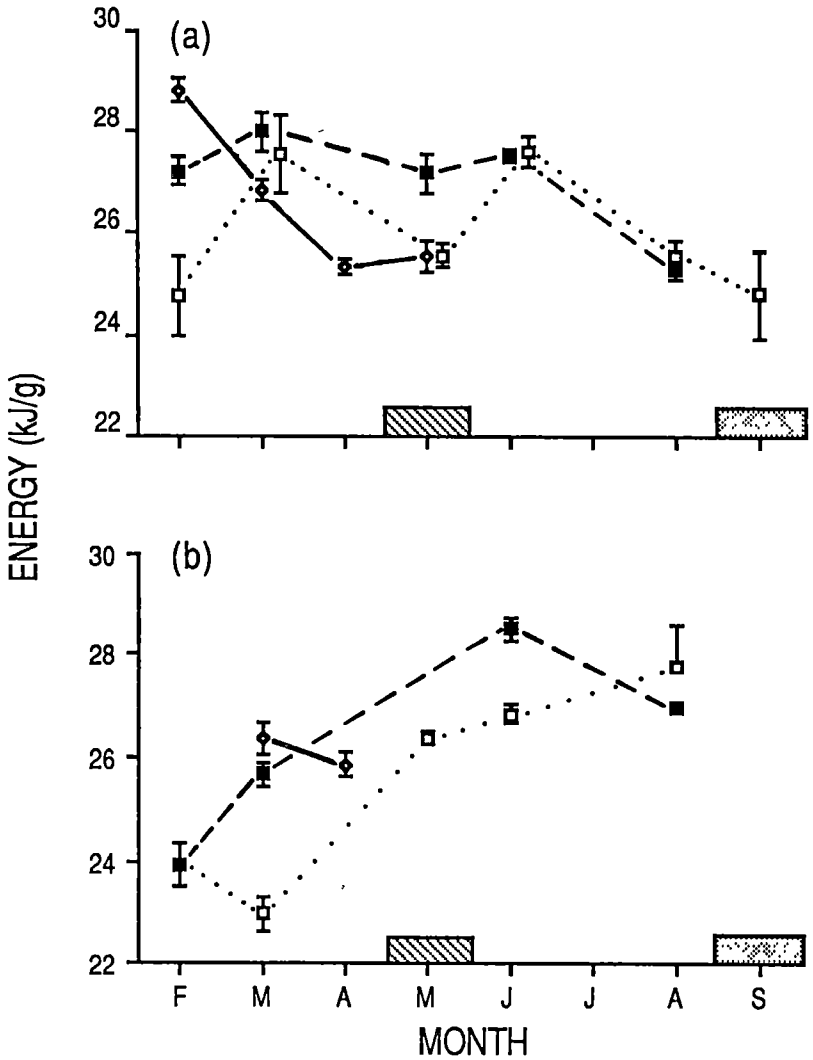


Fig. 4.11 Gonadal energy ±SE of a standard (a) female and (b) male fish from FLC (diamonds), CL (closed squares) and IL (open squares). Stippled boxes indicate spawning time of lake fish; hatched boxes indicate spawning time of creek fish.

increased during maturation (Fig. 4.11.b), whilst data for FLC males were minimal and no conclusions regarding them can be drawn. The relative energy content of testes of spawning males did not differ between localities and furthermore the relative energy content of ovaries and testes at this time did not differ within any locality.

Reproductive investment, expressed as the relative amount of energy invested in the gonad compared with the whole fish, is shown in Fig. 4.12. Regression equations for total body energy versus gonadal energy are given in Appendix 4a for females and Appendix 4b for males. At their respective spawning times, of the total energy within the bodies of a standard FLC female, *c.* 39% was devoted to gonad, whilst a standard CL female devoted *c.* 43% of its total energy to gonad (Fig. 4.12.a). Reproductive investment of a standard IL female reached a peak a month prior to spawning at *c.* 47%. At spawning time a standard FLC male allocated *c.* 27%, a CL male allocated *c.* 25% and a IL male allocated *c.* 32% of its total energy to gonad (Fig. 4.12.b). Females devoted a larger amount of their total energy to gonads prior to spawning than males (ANCOVA - April FLC: ns for slopes, for elevations $df_{13,1}$, F=18.73; June & August CL: for slopes $df_{20,1}$, F=25.36, $p<0.001$; August IL: ns for slopes, for elevatios $df_{23,1}$, F=24.26, $p<0.001$).

Figure 4.13.a shows least-squares lines of best fit for reproductive investment in stage V (ripe) females from the three populations obtained from regressions of total gonadal energy against total body energy. Similar relationships were apparent for lake females, which were both significantly different in slopes from the relationship for FLC females (ANCOVA - FLC/CL: $df_{14,1}$, F=7.05, $p<0.05$; FLC/IL: $df_{28,1}$, F=9.54, $p<0.01$). Lines of best fit for gonadal energy versus total body energy for stage V males are shown in Fig. 4.13.b. The only difference found between regression equations was for CL and IL males (ANCOVA - for slopes $df_{16,1}$, F=13.10, $p<0.01$).

4.3.10 Relationships Between Variables

It was suggested earlier that an inverse relationship existed between the water content and fat content of somatic tissues. Regressions of percentage fat by dry weight of somatic tissues against percentage water of somatic tissues for individual reproductive fish of each sex revealed significant correlations between these variables (Table 4.9) and Fig. 4.14 is an example of one of these relationshipsfor IL females pooling May, June and August samples.

In most cases significant relationships also existed for regressions of percentage protein and percentage water of somatic tissues for individual reproductive fish (Table 4.10), however, the percentage variation explained by the regression lines (r^2) were generally low. In only four of six cases were there significant relationships between percentage ash and percentage water of somatic tissues for reproductive fish; all significant relationships coming from lakes (Table 4.11). Regression lines generally explained only a small amount of the variance (r^2) for these data sets. There were no significant relationships between any other pairs of variables.

In an attempt to determine whether the gonad was being supplied with fat from the somatic tissues during maturation of fish, percentage fat by dry weight of the soma was regressed against absolute weight of fat in the gonad. In only three cases, all for IL females, were there significant negative

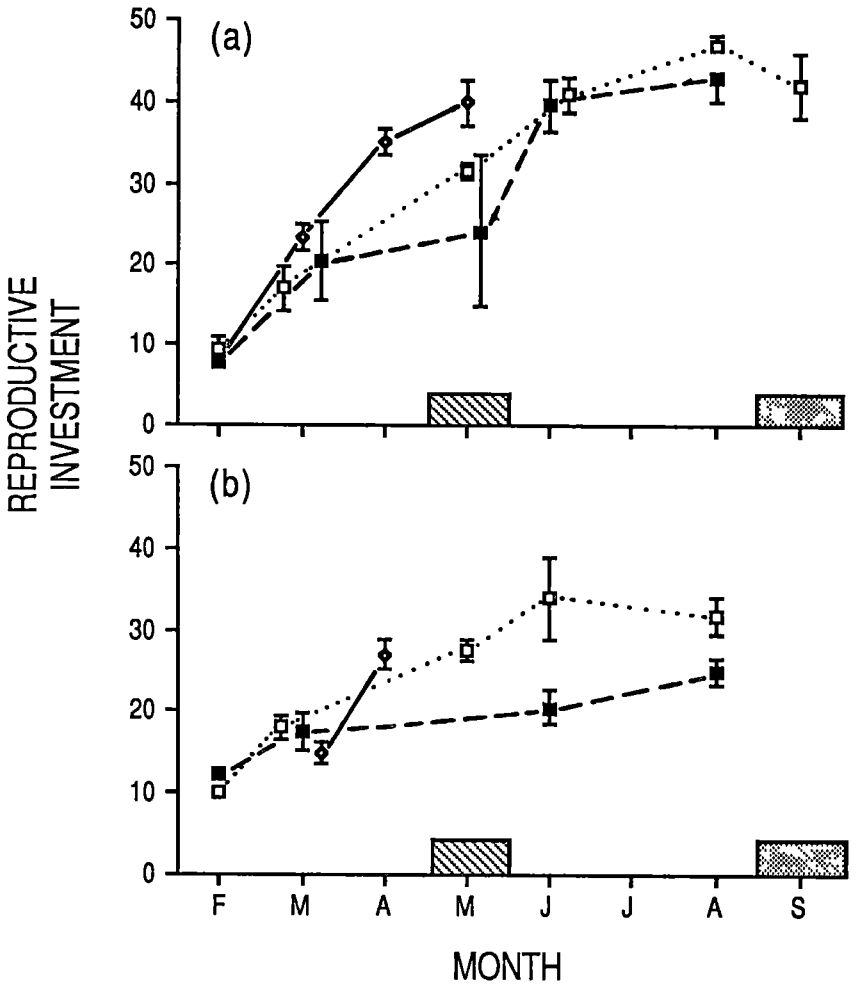


Fig. 4.12 Reproductive investment ±SE expressed as the percentage of total body energy (soma + gonad) devoted to the gonad of a standard (a) female and (b) male fish from FLC (diamonds), CL (closed squares) and IL (open squares). Stippled boxes indicate spawning time of lake fish; hatched boxes indicate spawning time of creek fish.

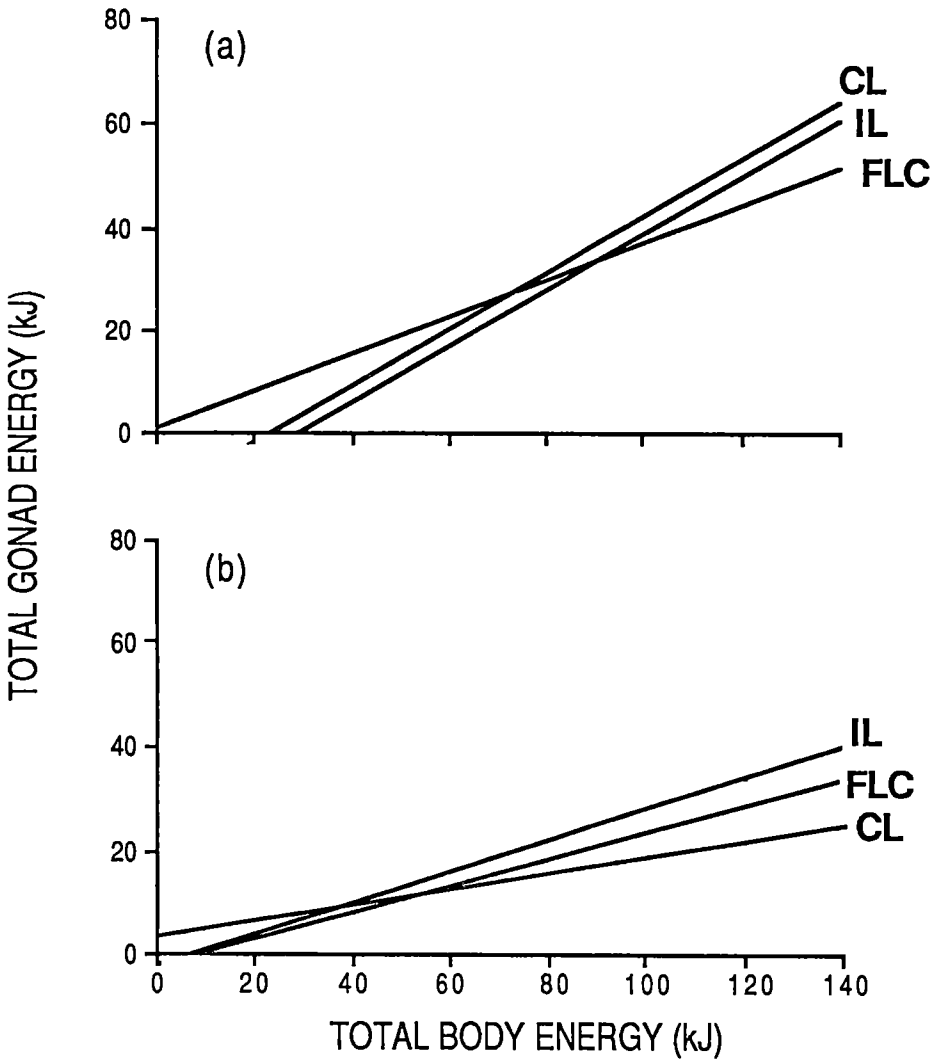


Fig. 4.13 Regression lines describing relationships between total gonadal energy and total body energy (gonad + soma) for Stage V (ripe) (a) females and (b) males from FLC. CL and IL.

Table 4.9 Regressions of somatic fat (% dry weight) against somatic water for fish at stages III through V (Equations are in the form y=bx+c where %fat is the dependent variable; data were arcsin transformed)

Locality	Sex	n	r^2	F	Probability	b	c
FLC	F	44	0.52	48.09	<0.001	-1.638847	1.723645
	M	28	0.81	99.15	<0.001	-1.888721	1.976123
CL	F	52	0.59	71.65	<0.001	-1.538951	1.690348
	M	26	0.26	8.29	<0.01	-1.140757	1.311064
IL	F	74	0.57	96.87	<0.001	-2.766260	2.844779
	M	44	0.29	24.49	<0.001	-1.233878	1.381906

Table 4.10 Regressions of somatic protein (% dry weight) against somatic water for fish at stages III through V. (Equations are in the form y=bx+c where % protein is the dependent variable; data were arcsin transformed)

Locality	Sex	n	r^2	F	Probability	b	c
FLC	F	44	0.16	7.95	<0.01	1.131850	-0.334335
	M	28	0.44	19.94	<0.001	1.614222	-0.795608
CL	F	51	0.43	36.3	<0.001	1.251675	-0.508631
	M	26	0.5	23.92	<0.001	0.353524	-0.285954
IL	F	74	0.49	67.49	<0.001	2.260418	-1.447077
	M	44	0.11	4.52	<0.05	0.954700	-0.197318

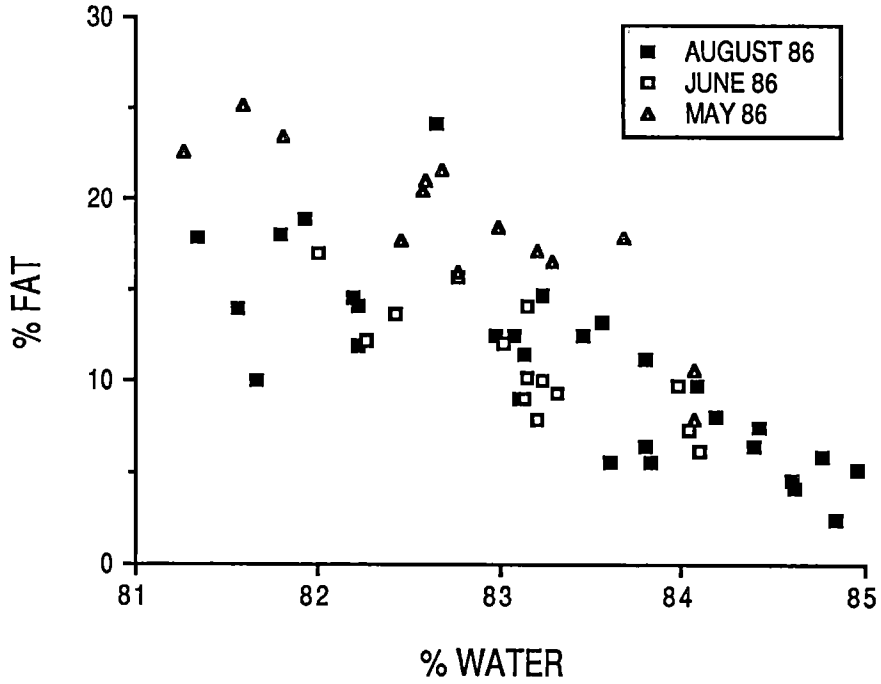


Fig 4.14 Plot of somatic fat (percentage by dry weight) with percentage somatic water for IL females from May, June and August 1986 samples.

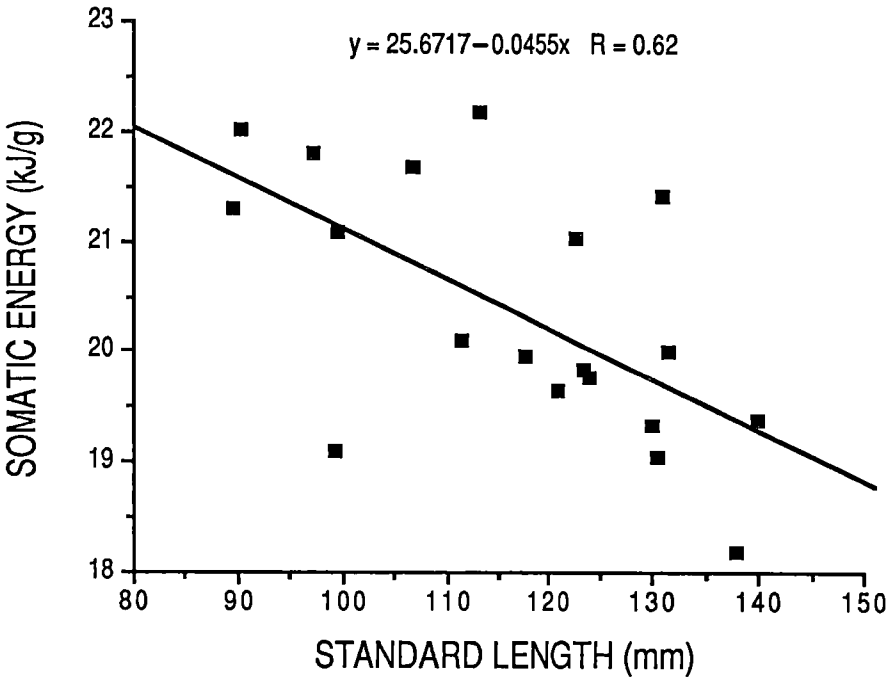


Fig. 4.15 Plot of standard length versus somatic energy of IL females for August 1986.

Table 4.11 Regressions of somatic ash (% dry weight) against somatic water for fish at stages III through V. (Equations are in the form y=bx+c where %ash is the dependent variable; data were arcsin transformed)

Locality	Sex	n	r^2	F	probability	b	c
FLC	F	44	–	–	>0.05	–	–
	M	28	–	–	>0.05	–	–
CL	F	52	0.25	16.77	<0.001	0.291382	-0.211936
	M	26	0.27	8.83	<0.01	0.353524	-0.285954
IL	F	74	0.36	40.37	<0.001	0.750458	-0.660892
	M	44	0.12	5.34	<0.05	0.204872	-0.143645

Table 4.12 Regression equations for percentage fat by dry weight of somatic tissue versus absolute weight of fat (g) in gonad, for individual IL females in May, June and August 1986. (Equations are in the form y=bx + c, where weight of gonadal fat is the dependent variable).

Month	n	r^2	Probability	b	c
v.86	6	0.77	<0.05	-0.554310	0.987520
vi.86	9	0.53	<0.05	-0.305010	0.925606
viii.86	19	0.41	<0.01	-0.650819	0.761517

correlations between percentage fat in the soma and weight of gonadal fat (Table. 4.12). The percentage variation explained by the regression lines decreased during maturation, suggesting either that fat was transferred from the gonad unequally among fish within a particular sample or that, as maturation progressed, gonadal fat was being supplemented from the diet.

4.3.11 Relationships of Constituents with Body Size

In several instances significant relationships were found between body size (standard length) and percentage constituents by dry weight of somatic tissues. The nature of the regression equations may provide some insight into the change in body composition over different sizes and consequently ages (see Section 3.3.9) of fish.

Table 4.13 shows all significant relationships between body size and body constituents. Body water was negatively correlated with body size in all cases for FLC and CL fish, however, positive correlations between these two variables were found for IL fish. Fat was always positively correlated with body size for CL fish while negative correlations between fat and body size were always found for IL females. FLC females showed negative correlations between body size and percentage fat, whilst FLC males showed positive correlations. In all but one case fish from the three localities showed negative correlations for protein and positive correlations for ash with body size.

Significant correlations between somatic energy and body size were less frequently encountered. FLC fish did not show significant relationships between body size and somatic energy, while CL females showed a positive correlation between these variables in December only. From February to September IL females had significant negative correlations between body size and somatic energy, and Fig. 4.15 illustrates one such relationship for August.

4.4. DISCUSSION

In the proximate analyses of *G. truttaceus* marked seasonal fluctuations of fat, protein, ash, water and energy in the somatic tissues were recorded. For both sexes the fat, and to a lesser extent, energy content, of somatic tissue increased to a maximum in late summer/early autumn (February/March), then decreased during maturation, reaching a minimum prior to spawning (May in creek fish, September in lake fish). Males and females generally showed similar patterns of fluctuations in body constituents, as has been found in other species (Medford and Mackay, 1978; Beamish *et. al.*, 1979; Beamish and Legrow, 1983), although in most cases during the present study, the magnitude of these fluctuations were different between sexes. For example, CL females reached a higher level of somatic fat than CL males. This may be due to more intense feeding by females in preparation for maturation, an occurrence which has been noted in *Coregonus albula* where females may feed by as much as 30% more than males at some times of the year (Dabrowski, 1982).

The somatic tissues of female *G. truttaceus* were depleted of fat and energy during maturation, to a greater degree than males, at all localities. Love (1980) suggested that this is a general rule for fish due to the greater energy requirements of ovaries compared with testes. The loss of fat and energy

Table 4.13 Regression equations for somatic fat, protein and ash (% dry weight) and percentage water against standard length for fish from FLC, CL and IL. Percentage data were arcsin transformed. (equations are of the form y=bx + c, where body constituents are the dependent variables)

Locality	Month	Body Constituent	n	Sex	r^2	F	Probability	b	c
FLC	vii.85	Fat	7	F	0.81	21.61	<0.01	-0.002065	0.249413
	viii.85	Ash	7	F	0.90	38.02	<0.01	0.002728	-0.188995
	ii.86	Fat	10	M	0.43	5.95	<0.05	0.001177	0.075804
	ii.86	Protein	10	M	0.50	8.00	<0.05	-0.001094	0.832992
	ii.86	Water	10	M	0.69	18.01	<0.01	-0.000850	1.026786
	iv.86	Fat	9	M	0.83	28.48	<0.01	0.004188	-0.206034
	iv.86	Protein	9	M	0.77	20.28	<0.01	-0.005718	1.215734
	iv.86	Water	9	M	0.85	35.00	<0.001	-0.001935	1.134606
	v.86	Fat	14	F	0.32	5.54	<0.05	-0.001753	0.246085
	v.86	Ash	14	F	0.39	7.71	<0.05	0.000987	0.009605
CL	x.85	Water	7	F	0.77	13.67	<0.05	-0.003293	1.281413
	xii.85	Fat	7	F	0.89	42.37	<0.01	0.009013	-0.722086
	xii.85	Protein	7	F	0.76	15.52	<0.05	-0.007541	1.497600
	xii.85	Water	7	F	0.73	13.81	<0.05	-0.003292	1.282284
	ii.86	Fat	6	M	0.90	39.39	<0.05	0.008936	-0.631251
	vi.86	Fat	6	M	0.9	37.85	<0.01	0.001490	0.085603
	vi.86	Protein	6	M	0.86	23.70	<0.01	-0.002813	0.998879
	viii.86	Ash	9	F	0.74	20.07	<0.01	0.001110	-0.026187
IL	vii.85	Ash	8	F	0.60	8.91	<0.05	0.000796	-0.029030
	xii.85	Ash	10	F	0.72	20.57	<0.01	0.001106	-0.053797
	ii.86	Ash	9	F	0.64	12.19	<0.05	0.000486	0.007882
	ii.86	Protein	9	M	0.69	15.62	<0.01	-0.005393	1.176462
	iii.86	Fat	7	F	0.70	11.86	<0.05	-0.004724	0.755793
	iii.86	Ash	7	F	0.62	8.32	<0.05	0.001199	-0.063684
	iii.86	Ash	7	M	0.59	7.31	<0.05	0.001295	-0.056742
	v.86	Fat	6	F	0.79	15.48	<0.05	-0.003366	0.538256
	v.86	Fat	6	M	0.83	14.83	<0.05	-0.008460	0.965485
	v.86	Protein	6	F	0.70	9.47	<0.05	0.002592	0.497984
	v.86	Ash	6	F	0.86	24.07	<0.01	0.000883	-0.033196
	v.86	Water	6	F	0.70	9.42	<0.05	0.001030	0.865405
	vi.86	Fat	9	F	0.68	14.91	<0.01	-0.001282	0.242428
	vi.86	Ash	9	F	0.62	11.45	<0.05	0.001208	-0.069708
	vi.86	Ash	5	M	0.86	18.19	<0.05	0.004740	-0.409128
	viii.86	Fat	19	F	0.53	18.94	<0.001	-0.001592	0.267828
	viii.86	Protein	19	F	0.25	5.52	<0.05	0.001061	0.686489
	viii.86	Ash	19	F	0.42	12.26	<0.01	0.001263	-0.051685
	viii.86	Water	19	F	0.49	16.32	<0.001	0.000718	0.910532

during maturation in female *G. truttaceus* can probably be explained in part by the transfer of fat to the gonads, as has been shown for other fish (Wootton and Mills, 1979; Wootton *et al.*, 1978; Dabrowski, 1982; Love, 1980; Newsome and Leduc, 1975) and, at least for lake fish, is due in part to a decline in feeding during winter. Overwintering, and a concomitant reduced feeding rate, has been shown to deplete the somatic fat content of the three-spined stickleback, *Gasterosteus aculeatus,* which had a minimum energy and fat content during winter and during breeding (Wootton *et al.*, 1978). Significant negative correlations between percentage somatic fat and weight of gonadal fat in IL females strongly suggests that depletion of somatic fat is partly due to gonad maturation. However, lack of correlation between these two variables among fish from other localities suggests that gonadal fat may also be supplemented from the diet.

The fact that non-reproductive *G. truttaceus* maintained relatively high levels of somatic fat and energy throughout the year further suggests that the loss of these constituents from reproductives of both sexes is due to reproductive activities. Peaks in somatic fat in lake non-reproductives coincided with zooplankton blooms in spring (pers. obs.), indicative of a productive time of the year. The weight and percentage of fat in testes of reproductive males was lower than in the ovaries of females. Males may, however, catabolise somatic fat for use in spawning behaviour, as has been shown for some teleosts (Dambergs, 1964; Shevchenko, 1972; Lapin, 1973) and the river lamprey, *Lampetra fluviatilis* (Heikkala *et al.*, 1984). For *G. truttaceus* it seems more likely that physiological, rather than behavioural processes influenced the use of fat, as depletion of somatic fat of males took place in the early stages of maturation as well as just prior to spawning.

Lake non-reproductives generally maintained greater somatic fat levels than creek non-reproductives throughout the year. The varying productivities of the different habitats and the availability of suitable food types for small fish, may account for this difference. An overall higher percentage fat and energy content of lake reproductives compared with those from the creek can probably be accounted for by the same argument.

Somatic protein and ash of reproductive *G. truttaceus* from CL both increased during summer and protein levels for both sexes at this localities decreased during maturation. Ash levels were higher for IL reproductive fish than non-reproductive fish at the spawning time of the former, although no detectable differences could be found between reproductives and non-reproductives at other localities. Several studies have shown ash to be high at spawning (Wootton and Mills, 1979; Wootton *et al.*, 1978; Beamish and Legrow, 1983) and Love (1970) has suggested that this may be due to either problems with osmoregulation during breeding or simply the depletion of other constituents causing a relative increase in ash. Seasonal fluctuations in ash levels may also reflect the flux of minerals and other inorganics for subsequent use in reproductive products. Somatic protein has been found to decrease during maturation in some species (Medford and Mackay, 1978; Beamish and Legrow, 1983; Heikkala *et al.*, 1984). Dawson and Grimm (1980) observed a 29% loss of protein over the spawning run of *Oncorhynchus nerka.* Many of these studies have been performed on large species which migrate long distances to spawn and may be subject to periods of starvation. It is not unexpected that such species may need to catabolise somatic protein for energy once lipid reserves

have been depleted (Heikkala *et al.*, 1984).

Some female *G. truttaceus* just prior to and during spawning had very low levels of somatic fat (between 2% and 5% by dry weight). In investigating the reasons for di sproportionate mortalities between sexes of *Perca flavescens,* Newsome and Leduc (1975) similarly found that some wild females after spawning had only 2% somatic fat by dry weight. They observed that, under laboratory conditions, starved fish died when they reached this level. It is possible that *G. truttaceus* may come close to minimum maintenance levels of fat and that such a depletion of their soma may have a detrimental affect on their survival. Lake fish may have to accumulate greater fat reserves than creek fish to survive the rigours of gonadal maturation and harsh winter conditions. If lake fish were to spawn in late autumn and so be in relatively poor condition during the winter, the further loss of somatic energy reserves might adversely affect their survival. It is possible, therefore, that there was and still is a selective pressure upon the adults to delay spawning until spring, so that energy reserves lost due to spawning can be replenished almost immediately.

It has long been known that a negative relationship exists between the fat and water content of fish. Love (1970) suggested that "fatty" fish have a water/fat line; *i.e.* that there is a negative correlation between somatic water and somatic fat. On the other hand "non-fatty" fish store most of their fat in the liver and so utilisation of fat reserves does not show up in the somatic tissue until the liver fat is exhausted. Many studies have shown a role for the liver in the synthesis and transfer of fats and proteins to the gonad (Medford and Mackay, 1978; Jangaard *et al.,* 1967; Wootton and Mills, 1979; Love, 1980); however, this was not investigated in the present study.

A water/protein line, where a negative correlation exists between water and protein, can also occur in fish (Love, 1970; Groves, 1970; Dambergs, 1964; Elliot, 1976) and has been demonstrated in this study for *G. truttaceus*. However, a stronger correlation was found between water and fat than between water and protein. It is therefore possible, to some extent, to predict both the fat and protein contents of somatic tissues from a knowledge of the water content, although the variation about the lines describing these relationships was considerable.

Reproductive females from IL were the only group which consistently showed significant relationships between percentage constituents by dry body weight and body size (standard length). The reason for this is uncertain. The small sample sizes involved in many of the analyses may contribute to the lack of consistency among populations regarding correlations between percentage constituents and body size. Craig (1977) found that older and larger *Perca fluviatilis* had greater somatic water than younger fish, which suggests a smaller percentage of fat as fish get older, and Love (1960) noted an increasing water content with age of spawning *Gadus callarius*. A similar occurrence was found for female *G. truttaceus* at IL. However, others working with different species have found that somatic fat content increases with increasing body size (Khawaja and Jafri, 1968; Groves, 1970; Love, 1970) and Love (1970) suggested that increasing fat reserves with increasing age is necessary to supply the proportionally larger gonad occurring in older and larger fish. It is necessary, when talking of body size to define whether body weight or length is being used. Body length is the preferable estimate of body size, as body weight will fluctuate seasonally,

depending upon its biochemical makeup which may be influenced by feeding (Elliot, 1976; Caulton and Bursell, 1977).

As a rule, ovaries contain a greater percentage of fat and testes a greater percentage of protein (Love, 1970). This was also the case for *G. truttaceus,* although in absolute terms, the female gonad was larger and therefore possessed a greater weight of both constituents. In some fish the proportion of fat and relative energy in ovaries and testes increases with maturation (Simpson, 1982; Heikkala, *et al.*, 1984), In others, as well as the present study, there is an increase of these constituents in ovaries, followed by a decrease in the period before spawning (Dabrowski, 1982, 1983). Dabrowski (1982) attributed this to the loss of the follicular membrane of the oocytes between maturity stages in female *Coregonus albula.*

Generally, testes have a higher percentage of water than ovaries (Love, 1970). In adult *Lampetra fluviatilis* water constituted between 55-57% of ovaries while it constituted between 85-87% of testes (Heikkala *et al.*, 1984). *G. truttaceus* testes similarly had a greater proportion of water than ovaries and also a greater proportion of ash. The gonads of lake males initially grew faster than those of females (Section 3.3.3), and water was accumulated by the testes at faster rate than in the ovaries. However, after the spawning of creek fish, the increases of the weights of the constituents in testes of lake males were minimal compared to females, suggesting an earlier maturation.

Female *G. truttaceus* invested more energy in reproduction as well as in the amount of fat and protein in their gonad than male *G. truttaceus.* Furthermore, lake females invested more energy in reproduction than FLC females. Greater amounts of fat and protein in the ovaries of lake females than in ovaries of FLC females probably explains the larger reproductive investment seen in the former. The larger reproductive investment for IL males than CL males is probably due to the slightly greater amount of fat and protein in the testes of IL males. Reproductive investment will be discussed in more detail in Chapter 5.

CHAPTER 5 GENERAL DISCUSSION

This study has demonstrated that considerable life history variation exists among four populations of *G. truttaceus* occurring in streams and lakes in Tasmania. Variation was found both within and between habitat types (stream and lake), although variation was greater between habitat types. Whilst life history variation occurred within both sexes variation between males from the four localities was less marked than for females. The most dramatic change associated with landlocking of the Carters Lake and Isabella Lagoon populations of *G. truttaceus* was the shift in spawning from the presumed ancestral time of early winter (as found in diadromous galaxiid species) to early spring; it is presumed that many of the other life history differences observed primarily result from this change. Gonad maturation patterns were similar at all localities for both male and female fish, however, lacustrine populations delayed spawning for about four months. This delay means that the time between reproduction and the commencement of the next maturation was considerably reduced compared with that of riverine fish. The shift in spawning time not only allowed the lacustrine females to continue depositing nutrients and energy into their gonads after riverine fish had spawned, but also allowed them to maintain relatively high somatic fat and energy reserves over the harsh winter months. Furthermore, spawning in early spring and the subsequent emergence of larvae approximately one month later, means that young fish experience favourable conditions within the lakes, when water temperatures are rising and a plentiful food resource of zooplankton exists.

The shift from the early winter spawning of diadromous *G. truttaceus* to the early spring spawning of landlocked *G. truttaceus* concurs with the pattern seen in the galaxiid family as a whole (see Appendix 5 for references for data on galaxiid life histories). In general, diadromous galaxiids spawn in late autumn/early winter and totally freshwater galaxiids spawn after winter (Table 5.1). Four apparent exceptions to this pattern occur. The landlocked species *G. pedderensis* has been found spent in late autumn, in tributaries of Lake Pedder in Tasmania and recent examination of mitochondrial DNA of *G. pedderensis* and *G. brevipinnis* (a diadromous species) suggests a very recent derivation of the former from the latter (J.R. Ovenden and R.W.G. White, pers. comm.). The lacustrine species *G. gracilis* from New Zealand is thought to spawn before winter, although this information is based on a single sample in March (McDowall, 1978b) and is considered insufficient for an accurate estimate of spawning time. Both *Neochanna apoda* and *N. diversus* spawn during late autumn/early winter after reviving from periods of aestivation (McDowall, 1970; Eldon, 1978). These last two species presumably take advantage of the best possible time at which to spawn in an unpredictable, often temporary habitat. Since spawning takes place soon after the inundation of their habitat by autumn rains, it appears likely that *N. apoda* and *N. diversus* spawn when the chances of their habitat drying up are minimal.

Landlocked *G. truttaceus* have altered their life histories in a similar fashion to that of other totally freshwater galaxiid species and furthermore, show a similar shift in spawning time to the landlocked population of *G. maculatus* in Lake Modewarre in Victoria (Pollard, 1971a). The reason or reasons why the shift has occurred in the lacustrine populations of *G. truttaceus* are by no means certain,

Table 5.1 Spawning times of diadromous and totally freshwater Australian and New Zealand galaxiids.

Spawning Time	Life History Group	
	Diadromous	Freshwater
Before Winter	*Galaxias truttaceus* *G. maculatus (Aus)* *G. brevipinnis (Aus)* *G. cleaveri* *G. argenteus* *G. fasciatus* *G. postvectis* *G. maculatus (NZ)* *G. brevipinnis (NZ)*	*G. pedderensis* *Neochanna apoda* *N. burrowsius* *G. gracilis ?*
After Winter		*G. olidus* *G. johnstoni* *G. fontanus* *G. auratus* *G. tanycephalus* *G. parvus* *G. occidentalis* *G. rostratus* *Paragalaxias dissimilis* *P. eleotroides* *Galaxiella pusilla* *Galaxias vulgaris* *G. divergens* *G. paucispondylus* *G. prognathus* *N. burrowsius*

however, a rapid decline in temperatures in the lakes in early winter may prevent spawning at this time. Riverine *G. truttaceus* experience a less dramatic drop in water temperature, followed by several weeks when the temperature remains relatively stable, and it is at this time that they spawn. McDowall (1970) has suggested that, in their evolution, many of the New Zealand freshwater galaxiids probably became cold adapted during the heavily glaciated Pleistocene. Freshwater galaxiids may similarly have been prevented from spawning at the same time as their diadromous ancestors for temperature reasons, although it is possible that selection in favour of post-winter spawners may have eliminated all others. In the present study lacustrine fish, by delaying spawning until after winter, maintained higher somatic fat and energy levels, which would presumably be an advantage in such a harsh environment. Selective processes delaying spawning would require individuals to spawn in asynchrony with the rest of the population; a trait that would be disadvantageous in any other situation. Therefore it may be that a simple environmental factor was the cause for the shift in spawning time. In the present study no lacustrine *G. truttaceus* were found to have spawned before winter and no riverine *G. truttaceus* were found not to have spawned before winter. A population of landlocked riverine *G. truttaceus* in Western Australia spawns before winter (R.W.G. White, pers. comm.). Temperatures are higher in the Western Australian streams where these fish are found and winter conditions are probably more benign than in the Tasmanian alpine lakes. Furthermore, a population of *G. truttaceus* inhabiting a coastal lagoon on the west coast of Tasmania is thought to spawn before winter (W. Fulton, pers. comm.).

Many studies have sought to determine the endogenous and exogenous factors involved in maturation and spawning (for reviews see de Vlaming, 1972; Scott, 1979; Bye, 1984), and most have dealt with the effects of photoperiod and temperature. Temperature appears to affect spawning time as well as the length of the spawning period in several fish species (Scott, 1979). Spawning seasons tend to become longer in marine fish in a southerly direction along the Atlantic coast of North America and some tropical species may spawn over many months (Scott, 1979). At equivalent latitudes marine species have longer spawning seasons than freshwater species and this has been attributed to the relatively stable, mild and productive nature of the marine environment (Scott, 1979; Bye, 1984). Furthermore, a shift in spawning time has been noted in several families of freshwater fish due to temperature differences (Fatio, 1882).

The shift in spawning time allowed lacustrine female *G. truttaceus* to maintain gonad growth and to make a larger overall investment in reproduction. This was accomplished partly by the use of greater accumulations of somatic fat and energy reserves and, presumably, partly by supplementation of gonad nutrients directly from the diet. However, the reproductive investments for lacustrine females were apportioned differently within Carters Lake and Isabella Lagoon populations. Isabella Lagoon females produced larger eggs in both years, whilst Carters Lake females produced a proportionally greater number of eggs with increasing body size in at least the second year of study. The pattern in egg size shown for diadromous and landlocked *G. truttaceus* in the present study is not wholly consistent with that seen within the galaxiid family.

The mean size of eggs of totally freshwater galaxiid species is significantly greater than that of

diadromous species ($\bar{x}_{freshwater}$=1.66 mm, $\bar{x}_{diadromous}$=1.28 mm, df_{17}, t=1.91, $p<0.05$) and there is no correlation between egg size and body size (Fig. 5.1). Body size is taken as the mean length of fish from various collections (see Appendix 5 for references from which these data were taken) and gives the same result as maximum length. Egg sizes of totally freshwater galaxiids are variable and range from 0.7 mm in *G. gracilis* (McDowall, 1978b) to 2.5 mm in *N. burrowsius* (Eldon, 1979b), whilst egg sizes of diadromous species only range from 1.0 to 1.5 mm.

A larger egg size in *G. truttaceus* from Isabella Lagoon is thought to be largely an environmentally induced variation, attributable to a delay in spawning. Nevertheless, genes for plasticity in egg size must initially exist. It is probable that the larger egg size found for Isabella Lagoon fish is adaptive, but it is likely that it did not initially occur by selective processes. Although the concept of optimum egg size has existed for some time now, other studies have shown its lack of wide applicability and have stressed the relative importance of proximal factors (Kaplan, 1980). If a fish normally breeds at a specific time during the year and breeding is delayed, a 'shut-off' mechanism may not exist to prevent any further supply of nutrients to the gonad. If this continued supply does occur, the limit to reproductive investment may be that of energy reserves within the body of the fish or within the environment itself *via* the diet or even body volume. Environmental factors affecting egg size may initially cause an increase, but if there is an advantage in having a larger egg, selection may act to maintain individuals with genes for this plasticity.

The adaptive significance of larger egg size in colder environments, such as probably existed when many galaxiid species became adapted to freshwater in New Zealand and Tasmania (McDowall, 1970), has long been established (Ware, 1975). Wootton (1984) demonstrated a correlation between egg size and spawning month in marine fish (implying a negative correlation with temperature) and several studies have indicated that egg sizes reflect optimal larval sizes which are designed to take advantage of the normal size spectrum of particles found in the environment (Cushing, 1969; Ware, 1977; Wootton, 1979). Blaxter and Hempel (1963) and Bagenal (1969) showed that larger larvae were produced from larger eggs for *Clupea harengus* and *Salmo trutta* respectively, an occurrence also demonstrated for *G. truttaceus*. Furthermore, they demonstrated that larger larvae were able to survive longer periods of starvation than smaller larvae and that their larger gapes allowed them to take advantage of greater ranges of food particles. In colder regions incubation periods of eggs tend to be longer than in warmer regions, due to the slowing of metabolic processes. Larger eggs tend to be produced in cold areas and a negative relationship has been found between egg size and egg mortality (Ware, 1975). McDowall (1970) suggested that larger larvae from larger eggs in totally freshwater galaxiids may have an advantage in being able to maintain their position in streams. It may also provide them with greater yolk reserves so that they can grow to a larger size to take advantage of food types characteristic of the lotic environment. Maintenance of position in streams appears unlikely to be the major reason behind egg size variation in *G. truttaceus* and other totally freshwater galaxiids, as it is not consistent with the larger egg sizes of some lacustrine populations and species.

In the present study intra-specific variation in fecundity within *G. truttaceus* did not follow the same pattern seen within the galaxiid family as a whole. Although the mean fecundity for diadromous

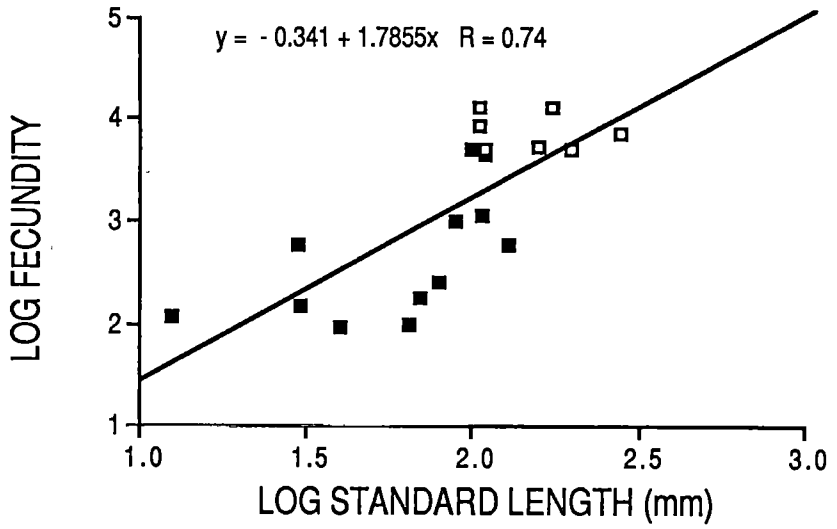


Fig. 5.2 Plot of log fecundity versus log standard length for totally freshwater galaxiids (closed squares) and diadromous galaxiids (open squares).

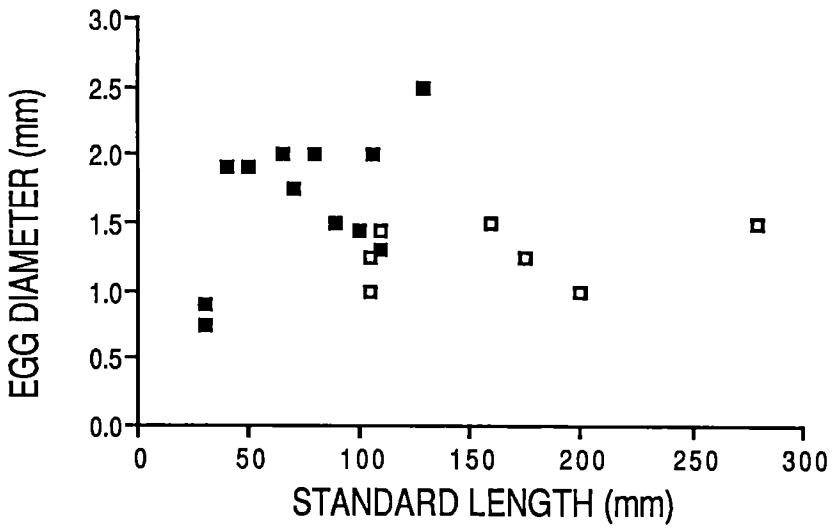


Fig. 5.1 Plot of egg diameter versus standard length for totally freshwater galaxiids (closed squares) and diadromous galaxiids (open squares).

galaxiids is greater than that of totally freshwater galaxiids ($\bar{x}_{diadromous}$=8107, $\bar{x}_{freshwater}$=1144, df_{17}, t=5.77, p<0.001), when the fecundity of all species is plotted against body size on a logarithmic scale, a linear relationship results (Fig. 5.2). Mean fecundity was taken as the average fecundity of all fish from collections cited in Appendix 5 and is only a rough estimate of this trait. A significant relationship did not exist for diadromous galaxiids alone so separate regressions cannot be compared. However, it does appear that fecundity is highly dependent upon body size and that the difference seen between mean fecundities is mainly a function of different body sizes between the two groups ($\bar{x}_{dadromous}$=162.1 mm, $\bar{x}_{freshwater}$=75.2 mm, df_{17}, t=3.92, p<0.01).

Egg number increased from an initial low value more rapidly with body size at CartersLake than at Fortescue Lagoon Creek or Isabella Lagoon; probably explaining the high reproductive investment shown for the Carters Lake population. This indicates that larger, and therefore older, Carters Lake *G. truttaceus* produce proportionally larger numbers of eggs and have a proportionally greater reproductive investment than smaller and younger fish. This may be due to predation both on juvenile and adult stages of *G. truttaceus* by *Salmo trutta* and *S. gairdneri* ; Carters Lake being the only adult habitat in the present study supporting large numbers of predators. It has been suggested, within the concept of 'bet-hedging', that animals produce greater numbers of offspring in response to heavy predation or mortality at the juvenile stage (Stearns 1977; Reznick and Endler, 1982). It has also been hypothesised that reproductive investment of a particular adult size class will increase as the risk of mortality in that class increases (Gadgil and Bossert, 1970; Michod, 1979; Charlesworth, 1980; Reznick and Endler, 1982). There is no concrete evidence to indicate which age class of the Carters Lake population is being predated upon the most. However, from length-frequency histograms for samples from Carters Lake it is thought that the majority of predation on post-larval stages of *G. truttaceus* has been on fish older than age one, although there were only small numbers of fish within all size classes from this locality. If the risk of mortality due to predation of adults increases with age, then this could explain the large increase in both reproductive investment and egg number in larger fish. A thorough investigation of the size classes of juvenile and adult fish being predated upon in Carters Lake may shed some light on this idea.

Several studies have shown a positive relationship between ration and fecundity in fish (Bagenal, 1969; Wilkinson and Jones, 1977; Hester, 1964), birds (Winkler, 1985) and reptiles (Dunham, 1982; Seigel and Fitch, 1985). The productivity of Carters Lake was thought to be poorer than Isabella Lagoon as the latter possessed extensive weed beds which support an abundant invertebrate fauna. Furthermore, females from both lake populations showed similar relationships between gonad weight and body weight and between gonad energy and body energy. The difference in reproductive investment was in the way the gonad weight and energy were apportioned between egg size and number. Although it appears unlikely, therefore, that the differences in fecundities between lake populations were due to different rations, Carters Lake females did possess larger somatic fat and energy reserves at the commencement of gonadal maturation than did Isabella Lagoon and Fortescue Lagoon Creek females.

As stated above, the mean body size of diadromous galaxiids is greater than that of totally

freshwater galaxiids. Isabella Lagoon *G. truttaceus* and, to a lesser extent, Carters Lake *G. truttaceus*, grew faster and were larger than diadromous *G. truttaceus* until about age 4, so body size variation seen inter-specifically cannot be extrapolated to this particular intra-specific study. Age at maturity and size at maturity are often highly correlated in animal species (Tinkle and Ballinger, 1972; Policansky, 1983). Fish maturing in their first year tend to be small for the simple reason that they have had only one year in which to grow, but more importantly, they have had to devote energy to both reproduction and growth in that year. Those galaxiid species known to mature and spawn in their first year, such as *Galaxiella pusilla* (Humphries, 1986), *Galaxias gracilis* (McDowall, 1970), *G. divergens* (Hopkins, 1971), *Paragalaxias dissimilis*, *P. eleotroides* (Fulton, 1982) and *Neochanna burrowsius* (Eldon, 1979b) tend to be small. On the other hand, those galaxiids known to mature and spawn in their second year or older, such as *G. truttaceus* (this study), *G. fasciatus* (Hopkins, 1979a) and *G. argenteus* (McDowall, 1978b), tend to be larger. The diadromous *G. maculatus* spawns at age 1, but is intermediate in size between the above two groups. McDowall (1972) has reported the occurrence of several dwarfed populations of *G. maculatus* in New Zealand lakes. These populations reach maturity as small as 30 mm, compared with the usual 70-80 mm of diadromous populations. Both forms of *G. maculatus* mature in their first year and are thought to be essentially annual.

The smaller mean size of totally freshwater galaxiids may be due to the preponderance of early maturers in this group, although there is evidently another factor involved, from the evidence of the diadromous and dwarfed populations of *G. maculatus* in New Zealand. The reasons for early maturity should nevertheless be investigated and related to body size and fecundity, rather than simply looking at the latter traits separately.

A large body of literature exists dealing with the concept of age and size at maturity and their adaptive natures (see McPhail, 1977). There is, however, no consensus concerning the selective processes involved in being at a particular size or age at maturity. Inherent in size and age at maturity is the trade-off between growth and reproduction, if reproductive investment increases disproportionately with body size and age (Reznick, 1983). This concept suggests that an organism which invests energy in current reproduction will do so at the expense of somatic growth and therefore its future reproductive output will be reduced. Alternatively, an organism which delays maturation may lose one breeding opportunity, but will enhance its reproductive output in the next breeding season from being at a larger size. Reznick (1983) found that energy that had been previously 'allocated' to reproduction in *Poecilia reticulata,* which had been prevented from breeding, could not be used for growth and was effectively 'lost' to the fish. However, this 'lost' energy was stored as visceral fat and could probably have been used in a future reproductive episode.

Why the preponderance of early maturity in totally freshwater galaxiids? The bet-hedging theory predicts that early maturity will be favoured in environments where there is low or unpredictable adult survivorship (Stearns, 1977; Dunham, 1982). There is reason to believe that this could have been the case in the evolution of freshwater galaxiids from ancestral diadromous stocks. It has already been stated that the probable origin of the New Zealand freswater galaxiid fauna may have been prior to or

during the last glaciation (McDowall, 1970). Conditions were colder then than now and harsh winters may have taken their toll of adults, making it advantageous to mature earlier so as to ensure at least one successful breeding episode. According to the bet-hedging theory, these conditions should also have selected for a large reproductive investment early in life and a short life span. Reproductive investment cannot be adequately assessed for galaxiids and early maturity is by no means the rule in totally freshwater species of this family, although several species do show small size, early maturity and relatively short life spans.

Mortality did not appear to be higher amongst Isabella Lagoon fish than those from the other localities, although temperatures over winter in this lake were certainly more extreme than temperatures in the streams. In fact the largest and oldest fish collected during the study were found at Isabella Lagoon. Some males from both lakes did mature in their first year of life, an occurrence which was not found in either stream population. A suggestion that this is an initial shift to an earlier maturity would be premature and, furthermore, the shift would be expected in females as well as males.

A trade-off between egg size and fecundity appears to exist within the galaxiid family when fecundity is corrected for the effects of body size by the use of residuals (Fig. 5.3). However, this was not found to be the case within populations of *G. truttaceus* during the present study. This suggests that resources or body size are in some way limiting, demonstrable only at a higher taxonomic level, so that one trait can only increase at the expense of the other. In his analysis of the life histories of reptiles and mammals Stearns (1983, 1984) found that many traits were dependent upon body size and that these trends were sometimes obscured at the intra-specific level. Hutchings and Morris (1985) showed that salmonid life histories were highly related to taxonomic groupings and that size alone did not effect patterns of covariation in life history traits.

Although it has already been argued that the differences in fecundities between diadromous and totally freshwater galaxiids are probably largely functions of body size, this does not imply that there is no relationship between egg size and number. In most fish species absolute fecundity and egg size increase with age and body size, however, when allowance is made for body size there is often a negative relationship between egg size and number (Mann and Mills, 1979). The adaptive significance of larger eggs in a cold or variable environment has been established previously in this chapter. Totally freshwater galaxiids became adapted to freshwater where there were few predators compared with the marine environment. The predation pressure on larvae and juveniles was presumably less in freshwater for this reason. Therefore the necessity for large numbers of young to offset intense predation during the vulnerable marine phase of diadromous galaxiids may have become redundant. If selection pressures limit the overall reproductive investment of an individual, as is thought to be the case (Mann and Mills, 1979), and assuming that large egg size is an advantage and that higher fecundity is not, then there may be selection in favour of retention of the former at the expense of the latter.

An obvious extension to the present study would be to investigate further the relative contributions of the environment and genes to the variation in life history traits among populations of *G. truttaceus*.

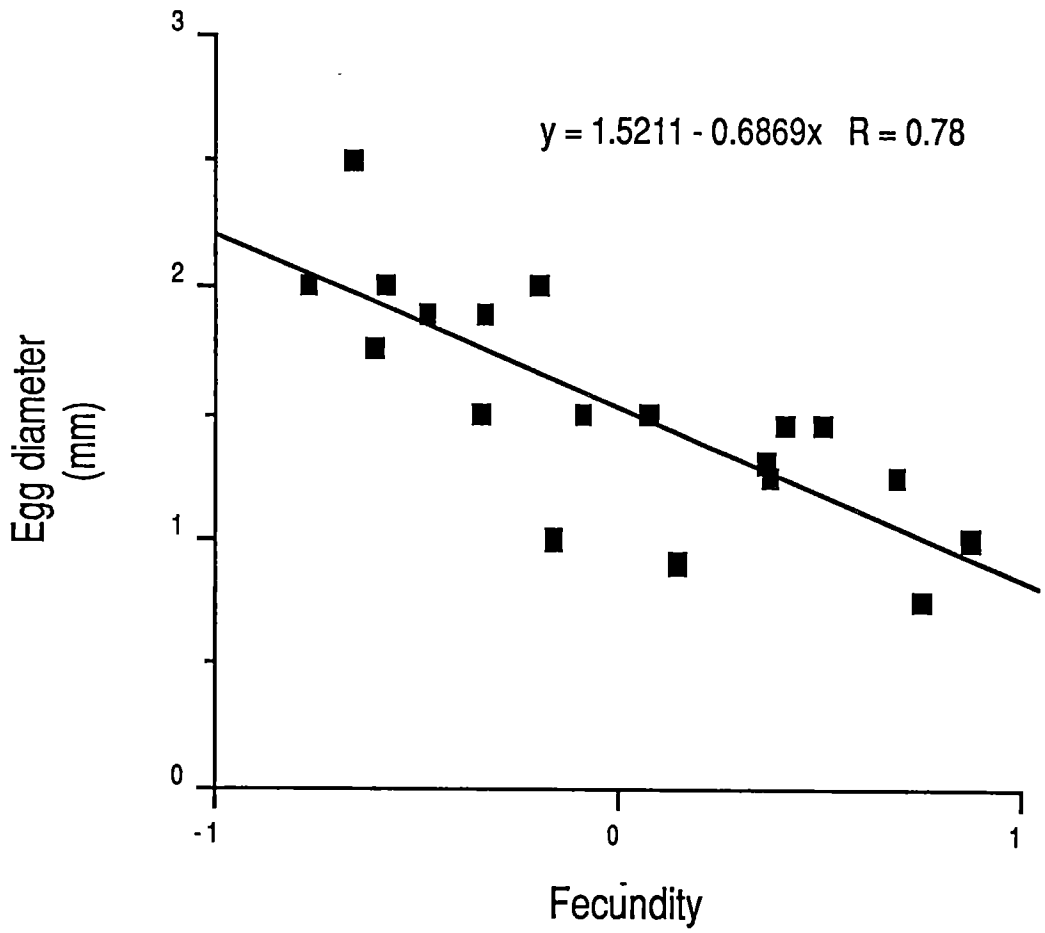


Fig. 5.3 Plot of size-corrected fecundity versus egg diameters for pooled diadromous and totally freshwater galaxiids. Fecundity was corrected for size by use of the residuals of regression of fecundity with body size.

There was an indication of a certain degree of plasticity in life history traits from the results of the maintenance of fish in the laboratory for one year. If nothing else, it demonstrated that under unsuitable conditions fish will not commence maturation, but will instead store energy in the form of visceral and somatic fat, presumably for use in the future. It would also be informative to try to induce spawning in lacustrine *G. truttaceus* at the time of normal spawning of riverine *G. truttaceus*, in an attempt to determine the extent of flexibility in spawning time. However, the best and most rigorous results would be gained if an extensive transplanting operation was performed, whereby several hundred stream fish were marked and released in a lake and *vice versa*. Sampled at the end of a specific period of time, the traits of the transplanted fish would indicate whether there were greater similarities with the traits of fish from their habitat of origin or from fish within their new habitat. Furthermore, if a successful breeding programme could be established and fish from a number of populations maintained over several generations under laboratory conditions, this would provide reliable evidence concerning the nature of life history variation within *G. truttaceus*.

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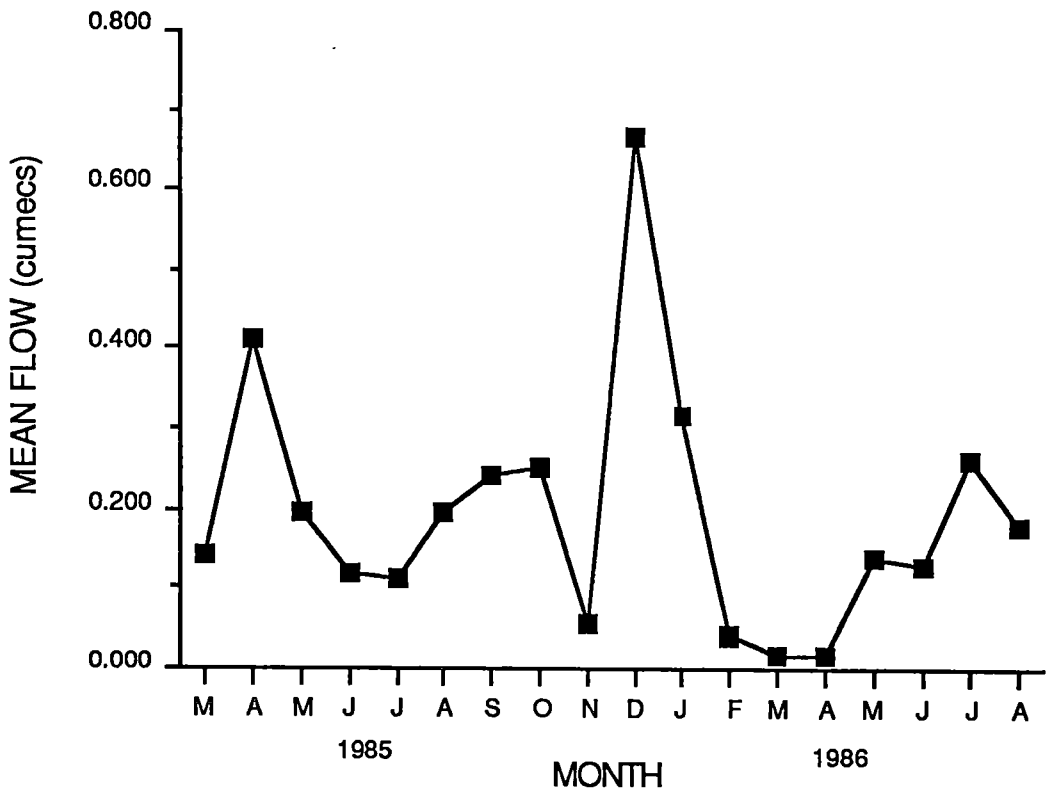
Appendix 1a Equations for regressions of gonad weight against body weight for maturity stages I through VI for females from FLC, AC, CL and IL. Equations are of the form $y = bx + c$; where y is body weight, x is gonad weight and b and a are constants. n = sample size: * = $0.01 < p < 0.05$; ** = $0.001 < p < 0.01$; *** = $p < 0.001$.

Stage	Locality	n	r^2	Probability	b	c
I	FLC	24	0.32	**	0.009820	-0.016599
	AC	33	0.86	***	0.002143	0.001167
	CL	10	0.82	***	0.003401	-0.003352
	IL	--	--	ns	--	--
II	FLC	42	0.77	***	0.003400	-0.006257
	AC	41	0.77	***	0.003823	-0.006393
	CL	17	0.77	***	0.005434	-0.010915
	IL	10	0.91	***	0.004383	-0.001548
III	FLC	19	0.22	*	0.035419	-0.011024
	AC	--	--	ns	--	--
	CL	19	0.6	***	0.107497	-1.634124
	IL	23	0.53	***	0.095366	-1.211137
IV	FLC	24	0.84	***	0.157232	-0.433363
	AC	7	0.74	*	0.054998	0.781970
	CL	22	0.74	***	0.281599	-2.613400
	IL	27	0.83	***	0.280433	-1.391205
V	FLC	12	0.76	***	0.159745	0.451008
	AC	--	--	ns	--	--
	CL	20	0.89	***	0.345417	-2.074968
	IL	34	0.92	***	0.318488	-0.928180
VI	FLC	43	0.26	***	0.002805	0.029628
	AC	--	--	ns	--	--
	CL	6	0.78	*	0.011945	-0.067456
	IL	52	0.4	***	0.148816	-1.916344

Appendix 1b Equations for regressions of gonad weight against body weight for maturity stages I through VI for males from FLC, AC, CL and IL. Equations are of the form y = bx + c; where y is body weight, x is gonad weight and b and a are constants. n = sample size: * = 0.01< p < 0.05*; * = 0.001< p < 0.01 *** = p< 0.001.

Stage	Locality	n	r^2	Probabilty	b	c
I	FLC	14	0.35	*	0.000831	0.000008
	AC	16	0.79	***	0.000865	-0.000889
	CL	--	--	ns	--	--
	IL	--	--	ns	--	--
II	FLC	18	0.67	***	0.001740	-0.000785
	AC	11	0.94	***	0.003438	-0.011742
	CL	12	0.66	**	0.003145	-0.006505
	IL	17	0.96	***	0.005161	-0.014668
III	FLC	11	0.79	***	0.054747	0.035347
	AC	--	--	ns	--	--
	CL	--	--	ns	--	--
	IL	--	--	ns	--	--
IV	FLC	17	0.82	***	0.131145	0.070920
	AC	--	--	ns	--	--
	CL	22	0.87	***	0.132915	0.060544
	IL	25	0.77	***	0.208902	-0.926002
V	FLC	15	0.96	***	0.192425	0.332733
	AC	8	0.89	***	0.174324	0.101363
	CL	13	0.87	***	0.166691	-0.141301
	IL	28	0.81	***	0.171146	0.112287
VI	FLC	--	--	ns	--	--
	AC	--	--	ns	--	--
	CL	--	--	ns	--	--
	IL	13	0.29	**	0.071373	-0.188899



Appendix 2 Water flow at AC over the period March 1985 to August 1986.

Appendix 3.a Results of t-tests between sexes for percentage (dry weight) of gonadal fat for fish from FLC, CL and IL.

Locality	Date	Female $\bar{x}$±SE	Male $\bar{x}$±SE	df	t	probability
FLC						
	ii.86	37.69±0.79	–	–	–	–
	iii.86	30.49±0.63	11.73±0.51	12	20.04	<0.001
	iv.86	25.94±0.35	11.56±0.47	14	23.78	<0.001
	v.86	25.07±0.64	–	–	–	–
CL						
	ii.86	36.93±1.13	13.69±1.30	8	13.47	<0.001
	iii.86	33.84±0.65	12.17±0.67	10	20.69	<0.001
	v.86	30.85±1.07	–	–	–	–
	vi.86	31.40±0.41	18.42±0.64	8	17.20	<0.001
	viii.86	24.99±0.63	14.80±1.00	10	9.04	<0.001
	ix.86	20.49	–	–	–	–
IL						
	ii.86	27.20±1.70	22.54	4	1.12	>0.05
	iii.86	31.32±1.47	8.69±0.41	11	15.96	<0.001
	v.86	27.68±1.06	15.38±0.28	9	10.32	<0.001
	vi.86	30.13±0.75	13.28±0.29	12	16.08	<0.001
	viii.86	24.28±0.90	17.13±0.31	24	4.71	<0.001
	ix.86	22.37±2.27	–	–	–	–

Appendix 3.b Results of t-tests between sexes for percentage (dry weight) of gonadal protein for fish from FLC, CL and IL.

Locality	Date	Female $\bar{x}$±SE	Male $\bar{x}$±SE	df	t	probability
FLC						
	ii.86	58.93±0.61	–	–	–	–
	iii.86	62.24±0.72	91.92±0.47	12	26.53	<0.001
	iv.86	63.79±0.21	90.09±0.53	14	57.67	<0.001
	v.86	66.11	–	–	–	–
CL						
	ii.86	54.08±1.30	78.45±1.96	7	9.76	<0.001
	iii.86	62.35±2.05	88.30±0.17	9	9.33	<0.001
	v.86	63.39±0.65	–	–	–	–
	vi.86	63.80±0.24	89.88±0.44	9	54.01	<0.001
	viii.86	65.20±0.55	89.42±1.59	11	18.51	<0.001
IL						
	ii.86	61.18±1.59	67.67	4	1.83	>0.05
	iii.86	64.49±2.54	82.71±1.03	11	7.05	<0.001
	v.86	61.58±1.44	86.19±0.16	8	13.61	<0.001
	vi.86	66.47±0.50	91.38±0.66	12	30.26	<0.001
	viii.86	67.48±0.41	88.77±3.37	24	10.20	<0.001
	ix.86	67.52±0.31	–	–	–	–

Appendix 3.c Results of t-tests between sexes for percentage (dry weight) of gonadal ash for fish from FLC, CL and IL.

Locality	Date	Female $\bar{x}$±SE	Male $\bar{x}$±SE	df	t	probability
FLC						
	ii.86	4.66±0.73	–	–	–	–
	iii.86	2.74±0.18	7.32±0.13	12	16.57	<0.001
	iv.86	3.14±0.63	7.17±0.11	14	4.24	<0.01
	v.86	3.62±0.05	–	–	–	–
CL						
	ii.86	3.81±0.85	7.62±1.92	8	1.65	>0.05
	iii.86	3.73±0.60	5.87±1.07	10	1.91	>0.05
	v.86	2.30±0.09	–	–	–	–
	vi.86	3.72±0.08	6.84±0.27	9	11.98	<0.001
	viii.86	3.93±0.80	4.00±0.43	11	0.05	>0.05
						–
IL						
	ii.86	3.25±0.22	5.17	4	3.87	<0.05
	iii.86	3.80±0.42	6.36±0.87	11	2.5	<0.05
	v.86	3.71±0.12	9.08±0.93	8	5.73	<0.01
	vi.86	3.41±0.18	6.88±0.27	12	10.90	<0.001
	viii.86	5.47±0.98	7.13±0.11	24	1.02	>0.05
	ix.86	3.59±0.07	–	–	–	–
	viii.86	68.46±0.63	79.36±1.55	26	7.83	<0.001

Appendix 3.d Results of t-tests between sexes for percentage of gonadal water for fish from FLC, CL and IL.

Locality	Date	Female $\bar{x}$±SE	Male $\bar{x}$±SE	df	t	probability
FLC						
	x.85	87.79±0.43	89.09±1.05	11	1.07	>0.05
	xii.85	79.62±1.56	–	–	–	–
	ii.86	71.30±0.83	84.10±0.65	14	12.36	<0.001
	iii.86	66.86±0.75	82.43±0.32	22	15.46	<0.001
	iv.86	65.31±0.32	79.34±0.42	18	26.96	<0.001
	v.86	82.59±1.85	87.62±3.73	15	1.26	>0.05
CL						
	x.85	80.96±1.25	83.56	10	1.39	>0.05
	xii.85	86.85±1.00	–	–	–	–
	ii.86	72.24±1.99	81.07±0.42	11	4.03	<0.01
	iii.86	66.35±2.30	79.65±0.51	11	3.75	<0.01
	v.86	67.74±0.65	81.17±2.62	7	6.85	<0.001
	vi.86	68.97±0.29	77.09±0.92	10	8.41	<0.001
	viii.86	68.19±0.50	78.01±0.78	12	11.14	<0.001
	ix.86	72.40	82.05	–	–	–
						–
IL						
	x.85	82.26±1.92	82.24±1.76	9	0.01	>0.05
	xii.85	85.49±0.80	–	–	–	–
	ii.86	78.25±2.71	84.83±0.13	16	2.42	<0.05
	iii.86	71.68±1.90	82.22±0.24	12	5.5	<0.001
	v.86	67.89±0.40	78.72±0.23	10	23.39	<0.001
	vi.86	68.25±0.36	76.48±0.52	12	13.39	<0.001
	ix.86	80.32±2.72	85.53±1.06	17	1.41	>0.05

Appendix 4.a Regression equations for total body energy (soma + gonad) versus ovarian energy used to calculate values for reproductive investment for a standard fish having a total body energy of 110 kJ. Equations are of the form y=bx+c, where gonad energy is the dependent variable.

Locality	Month	n	r^2	F	Probability	b	c
FLC	ii.86	4	0.91	19.54	<0.05	0.171164	-9.203029
	iii.86	9	0.67	14.30	<0.01	0.242939	-1.089454
	iv.86	11	0.92	104.7	<0.001	0.354392	-0.342391
	v.86	3	–	–	ns	–	–
CL	ii.86	4	0.94	31.57	<0.05	0.086698	0.024509
	iii.86	7	0.79	18.86	<0.01	0.258146	-5.409508
	v.86	6	0.84	21.72	<0.01	0.625210	-41.842300
	vi.86	5	0.98	176.57	<0.001	0.554013	-17.465449
	viii.86	8	0.95	106.75	<0.001	0.574826	-16.227229
IL	ii.86	5	0.93	28.55	<0.05	0.135369	-4.668850
	iii.86	6	0.94	66.09	<0.01	0.263143	-10.146330
	v.86	6	0.99	1651.83	<0.001	0.441027	-13.79604
	vi.86	9	0.99	560.07	<0.001	0.553865	-15.74077
	viii.86	19	0.99	1198.16	<0.001	0.520093	-5.811231
	ix.86	4	–	–	ns	–	–

Appendix 4.b Regression equations for total body energy (soma + gonad) versus testicular energy used to calculate values for reproductive investment for a standard fish having a total body energy of 110 kJ. Equations are of the form y=bx+c, where gonad energy is the dependent variable.

Locality	Month	n	r^2	F	Probability	b	c
FLC	iii.86	5	0.90	26.26	<0.05	0.139630	1.167270
	iv.86	5	0.95	53.16	<0.01	0.257681	-1.326604
CL	ii.86	1	–	–	ns	–	–
	iii.86	7	0.92	60.04	<0.001	0.197140	-1.864022
	v.86	4	0.96	54.37	<0.05	0.293296	-1.778838
	vi.86	5	0.78	10.90	<0.05	0.486451	-16.136106
	viii.86	7	0.94	64.35	<0.01	0.390811	-7.986854
IL	ii.86	5	0.93	41.21	<0.01	0.124614	-0.282439
	iii.86	4	0.92	24.31	<0.05	0.158420	1.788368
	vi.86	5	0.78	10.90	<0.05	0.186760	2.132698
	viii.86	4	–	–	ns	–	–

Appendix 5 Sources from which data were taken in the analysis of galaxiid life history traits.

Galaxiid species	Source(s)
Galaxias brevipinnis	McDowall, 1970 Andrews, 1976 McDowall, 1978 McDowall and Frankenberg, 1981
G. olidus	McDowall and Frankenberg, 1981 Fletcher, 1979
G. johnstoni	McDowall and Frankenberg, 1981 Andrews, 1976
G. pedderensis	McDowall and Frankenberg, 1981
G. fontanus	McDowall and Frankenberg, 1981
G. truttaceus	McDowall and Frankenberg, 1981 Andrews, 1976 This study.
G. auratus	McDowall and Frankenberg, 1981 Andrews, 1976 Humphries (unpubl.)
G. tanycephalus	McDowall and Frankenberg, 1981 W. Fulton (pers. com.) Andrews, 1976 Humphries (unpubl.)
G. cleaveri	McDowall and Frankenberg, 1981 Andrews, 1976 Humphries (unpubl.)
G. parvus	McDowall and Frankenberg, 1981
G. maculatus	Burnet, 1965 McDowall, 1968 Benzie, 1968b McDowall, 1970 Pollard, 1971a Andrews, 1976
G. occidentalis	McDowall and Frankenberg, 1981
G. rostratus	McDowall and Frankenberg, 1981
G. argenteus	McDowall, 1970 McDowall, 1978
G. fasciatus	McDowall, 1970 McDowall, 1978

	Hopkins, 1978a Hopkins, 1978b Mitchell and Penlington, 1982
G. postvectis	McDowall, 1970 McDowall, 1978
G. vulgaris	Benzie, 1968b McDowall, 1970 Cadwallader, 1976 McDowall, 1978
G. gracilis	McDowall, 1970 McDowall, 1972 McDowall, 1978
G. divergens	McDowall, 1970 Hopkins, 1971 McDowall, 1978
G. paucisponylus	McDowall, 1978
G. prognathus	McDowall, 1970 McDowall, 1978
Galaxiella pusilla	Bachouse and Vanner, 1978 McDowall, 1978a Humphries, 1986
G. nigrostriata	McDowall and Frankenberg, 1981
G. munda	McDowall and Frankenberg, 1981
Neochanna apoda	McDowall, 1970 Eldon, 1978
N. burrowsius	McDowall, 1978 McDowall, 1970 McDowall, 1978 Eldon, 1979a Eldon, 1979b
N. diversus	McDowall, 1970 McDowall, 1978
Paragalaxias dissimilis	Fulton, 1982
P. eleotroides	Fulton, 1982
P. mesotes	Fulton, 1982
P. julianus	Fulton, 1982 Humphries and Davies (Unpubl.)
Brachygalaxias bullocki	Campos, 1972

"The terrified fish, with open gills and gold-filmed eyes, surrendered to death, or struggled and slithered in anguish to escape it, and, as often before, his heart was filled with pity for these fish and gloomy detestation of human beings. Why were these people so brutish, so raw, so unbelievably slow-witted? Had nobody eyes, neither men nor fish-wives, nor the cheapening burgesses around them? Why had they never seen these anguished gills, these eyes glazed, with the agony of death, these tail-fins, beating the air so wildly - or felt the bitter, desperate horror of this slithering fight against extinction, this last, unbearable transformation of lovely and mysterious fish, as a shiver ran along their dying bodies, and they lay, exhausted and limp, pitiful meals for the table of some gluttonous burgess? These people were all blind; nothing ever spoke to them or moved them. A poor, beautiful beast might die in front of them, or a master, in some saint's face, have revealed all the pain, the thought, the noble hopes, the dark, the clutching fear in a human life, making of it a visible shudder - it all meant nothing; they could not see."

Narziss and Golmund **by Hermann Hesse**